even an alarming degree of ileus after operation within the peritoneal cavity.

SUMMARY

1. The dosage of avertin should be individual.

2. Given in accordance with established principles avertin can be used with complete safety.

3. Drugs which depress respiration should not be given before avertin until the anæsthetist has gained full experience of its action. Larger doses of avertin are preferable in difficult subjects.

4. Liver and kidney disease are not contra-indications to its use, but call for reasoned dosage; nitrous oxide and oxygen is preferable to ether.

5. Avertin reduces post-operative morbidity and mortality.

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AN EXPERIMENTAL PRODUCTION OF CORONARY THROMBOSIS AND MYOCARDIAL FAILURE

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INTRODUCTION

UNDER normal conditions we may assume that the sympathetic and parasympathetic divisions of the autonomic nervous system, which are synergistic in function, are in equilibrium, and that any disturbance of the physiological balance of these nerves must result in physiological dysfunction and later in pathological changes of the dually innervated visceral organs.

Vagotonia and sympatheticotonia, classified as to the relative reactivity of the sympathetic and parasympathetic nerves, have long been recognized as clinical entities, but their relation to common as well as obscure symptom-complexes appears to have been neglected.

Physiological dysfunction of a group of central nuclei may in turn produce a symptom-complex, from an increase in functional activity in the respective divisions of the autonomic nervous system, which may affect the whole nervous system or only a special part of it. However, vagotonia and sympatheticotonia do not necessarily imply hyperirritability or physiological dysfunction of the nerve centres involved, but, we think, may be produced by an excess of stimulating substances in the blood.

The work here reported, begun in 1933, deals only with repeated intravenous injections of the stimulating substance in the blood which simulates parasympathetic activity, namely, acetylcholine.

METHODS AND MATERIALS

Concentrated solutions of acetylcholine iodide (Hoffman-La Roche) or acetylcholine bromide (Eastman) were prepared, placed in rubber-capped vials, and sterilized (1 c.c. = 25 mg. acetylcholine). For injection purposes a 1/10,000 solution was used, prepared by dissolving 2 c.c. of the concentrated solution with 500 c.c. of sterile normal saline. Each dog received this amount daily, regardless of his weight and physical condition.

The dogs were trained to lie on the right side and to maintain this position throughout the injection period of 90 minutes. Using a

Woodyatt pump, injections were made into the leg vein (No. 21 needle) at the rate of about 5.5 c.c. per minute. For this type of chronic survival experiment we found that a Marriotte cylinder with an air trap was more satisfactory, and consequently replaced the Woodyatt in all our later experiments (see diagram).

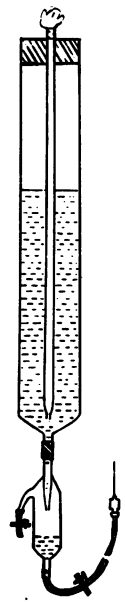

DIAGRAM.—Constant pressure cylinder as used in our continuous intravenous injections—modification of a Marriotte bottle.

In the hope of correlating any clinical symptoms with the experimental results, routine procedures were carried out on each dog. Initial values of the following were obtained over a period of at least one week prior to the commencement of the injection, and then routinely carried out each week.

1. Blood cultures and smears, red and white cell counts, viscosity and percentage hæmoglobin determinations.
2. Blood-pressure determinations, made by direct readings from the femoral artery (Dameshek and Loman, 1932), using, however, a filled system of freshly-boiled citrate solution.
3. In order to determine the effect of repeated flooding of the system by acetylcholine on the choline-esterase of the blood, estimations were made on the blood-serum, using a modification of Steadman's titration method (Lucas, Hall and Ettinger, 1935).
4. An endeavour was made to estimate each animal's exercise tolerance.
5. As a further check on the cardiac rate and condition electrocardiograms were taken. Three sets were taken on each dog previous to any injection. These served as normals for the individual dogs. Electrocardiograms were taken more frequently as the developing clinical condition of the animal became more serious.
6. Initial and weekly weighings were also taken of each dog.

The following daily routine was carried out.—

I. Before each experiment the animal was made to lie quietly for 10 minutes, and the resting heart and respiratory rates were recorded. Since in the unanæsthetized dog a continuous intravenous injection of acetylcholine in amounts up to 1 mg. per minute always causes acceleration of the heart, the heart-rate during injection was taken as an index of the action of the acetylcholine solution, and was recorded regularly by auscultation. During injections the heart-rate was taken every minute for the first five minutes and then every five minutes throughout the injection. Respiratory rates were taken every fifteen minutes.

II. Rectal temperatures were taken each day before injection. Room temperatures were also noted.

The experiments were carried out on 8 dogs arranged in two groups: A—young dogs (Nos. 4, 5, 6, 9); B—older dogs (Nos. 3, 8, 46, 47). These animals were injected daily, seven days in the week, until they died.

EXPERIMENTAL RESULTS

CARDIOVASCULAR

1. *Heart-rate.*

The resting heart-rate of the dog varies considerably from day to day, a normal for our dogs being about 80 to 100 per minute. Before the start of the injection the heart-rate of any individual dog for that day was quite constant. Within 10 to 20 seconds after the start of the injection the heart-rate always was greatly accelerated, even to two or three times the resting normal rate. Throughout the 90 minutes of injection the rate often varied considerably from the normal to 150 to 240, to 100 to 190, etc. for ½ minute counts, taken at five minute intervals by means of a stethoscope. Rates over 250 were frequently encountered but the beats could not be accurately counted. Some of the animals, after showing clinical signs of myocardial failure, developed a tolerance to acetylcholine to such an extent that there was either no, or a very slight irregular, increase in heart-rate with injection. In D 5, in whom this tolerance was very noticeable, the dyspnœa and other clinical signs seemed to be much more severe than in those dogs which showed only a slight tolerance. When the injection was completed and the needle withdrawn the heart-rate always decreased to almost the resting normal level within 20

seconds, and within 2 minutes had returned to the normal again.

Electrocardiograms taken synchronously with the start of the injection showed strikingly the very rapid increase in heart-rate, and, as well, the sudden decrease in heart-rate when the acetylcholine injection was stopped. That the cardiac acceleration by acetylcholine was not a volume-effect was shown by control experiments in which normal saline was injected under identical conditions.

2. *Heart-sounds.*

Following the heart-rate so consistently by auscultation it was easy to detect the development of systolic murmurs in every dog. So great is the difficulty in localizing cardiac murmurs in the dog that we made no attempt at their definite classification, other than to distinguish them as systolic. The murmurs at first were very slight and were not constantly present, but within a few days they became quite harsh, blowing in type, and constant throughout the day and night. Later, the murmurs became progressively rougher and louder, and eventually both first and second sounds were practically indistinguishable. The rapidity with which these murmurs developed varied greatly in the different dogs, in the old dog group being detected after 12 to 19 continuous daily injections. In the younger dogs the murmurs developed after 34 to 136 days (although one in 7 days).

Following the development of the systolic murmurs the clinical course was unchanged for many weeks except in one animal which developed extrasystoles and another, a partial heart-block.

All the animals showed terminal signs of cardiac failure, *viz.*, decreased exercise-tolerance, dyspnœa, râles and cyanosis. Diastolic murmurs developed coincidently. These signs became progressively more pronounced until the animals died.

The death of 3 animals occurred while under observation in the laboratory. The others died unexpectedly during the night. In those observed cases the animals showed signs of genuine cardiac distress, whimpered, gasped for breath, expelled some frothy fluid, became very cyanosed, unconscious and died. In one case the animal had two severe attacks, each lasting a minute or more, before the fatal attack. Each of the other two observed dogs had but the one attack, lasting about one minute.

3. *Electrocardiographic changes.*

In the old dogs which showed coronary arterial damage at autopsy the most serious early electrocardiographic changes were evident. In D 3 there were bouts of ventricular extrasystoles. D 8 showed a progressive prolongation of the P-R interval from the normal of 0.1 seconds to 0.4 seconds, and an occasional heart-block. Other dogs had slight prolongation of their P-R interval. Diphasic and inverted T waves were an inconstant finding, but, terminally, the T waves showed high voltage and a high take-off.

4. *Observations on blood-pressure during injections.*

Following the injection of acetylcholine in the unanæsthetized animal the blood-pressure fell rapidly from about 120 to 85 mm. Hg. and remained low. This was associated with a maintained increase in heart-rate, which was inversely proportional to the blood-pressure. There was no permanent change in blood-pressure as recorded weekly by the direct arterial method. During the actual injection periodic flushing of the skin was very common. In some cases the flushing persisted throughout the injection period. This peripheral vaso-dilatation was very marked about the mucous surfaces. When the injection was discontinued the peripheral vaso-dilatation disappeared and the blood-pressure returned to normal within 20 seconds.

5. *Esterase.*

The normal serum-esterase value* has a fairly narrow range for the same dog (Lucas, Hall and Ettinger, 1935). The continuous daily injection of 500 c.c. acetylcholine solution (= 50 mg. acetylcholine) did not produce any marked change in the esterase value from week to week throughout the life of the animals. This enzyme system appears to be very stable and efficient. Normally, very small quantities (0.06 to 0.6 mg. per litre) of acetylcholine are present in whole blood of the dog (Ettinger and Hall, 1934). On several occasions estimations of esterase were made from blood taken before,

* A unit has been defined in this laboratory as the concentration of enzyme which liberates 1.00 c.c. of 0.01 N acid when 0.50 c.c. of serum acts on 25 mg. of acetylcholine in a total initial volume of 11.5 c.c. at a pH of 8.0 and temperature of 37.5°.

during, and immediately following a daily injection of 500 c.c. acetylcholine solution. The values in these determinations were practically constant.

6. *Blood examination.*

Weekly values for the red blood-cell count, white blood-cell count, hæmoglobin and blood viscosity all remained within normal limits.

7. *Weight.*

The dogs were weighed individually each week. All dogs either maintained or increased their weight during the progress of the experiment until such time as their exercise-tolerance, general condition, appetite, etc., became poor.

8. *Blood cultures.*

Blood cultures on all dogs were repeatedly negative until the animals were in the final stages of cardiac failure, when the blood of 3 animals showed a terminal infection with *B. pyocyaneus*. In control experiments *B. pyocyaneus* caused no damage to the heart.

GASTRO-INTESTINAL SIGNS

1. *Salivary.*

During each injection-period throughout the experiments salivation was very profuse. In two of the dogs the volume of saliva secreted during the 90 minutes of injection totalled between 70 to 150 c.c. each. In those dogs which developed a marked tolerance to acetylcholine, as evidenced by very slight cardiac acceleration, the amount of salivary secretion did not diminish.

2. *Gastric and intestinal.*

Retching and vomiting were very common occurrences during the earlier injections. The vomitus was foul-smelling and contained much undigested food from the previous day's feeding. As the acetylcholine tolerance developed, retching and vomiting occurred much less frequently. Towards the end of each individual animal's experiment occasional hæmatemesis occurred. Diarrhœa was very common during the pre-tolerance stages of the experiment; later the stools were better formed. Blood-streaked stools and melæna were frequently observed, probably an effect of extreme mucosal vaso-dilatation.

AUTOPSY

Abdomen.

The animals were well nourished, with normal supplies of subcutaneous and omental fat. The intestines appeared bluer than normal, but glistening and shining. When the gastro-intestinal tract was removed and opened much blood-stained material was found. The gastric mucosa showed much congestion, and in places was partially absent. In the small intestine large, severely congested, patches were superimposed upon a generalized mild congestion of the whole submucosa. This congestion extended down to and often beyond the ileo-cæcal junction. The many scattered Peyer's patches, extending almost throughout the whole small bowel, appeared to be most affected.

The liver appeared dark and mottled, typical of chronic passive congestion. The kidneys were usually normal-looking, with the capsule stripping easily. On the cut surface the cortex and medulla were clearly defined. In one case, D 3, two large infarcted areas were noticed; the rest of the kidney substance was congested.

Thorax.

Heart.—The pericardium was usually smooth and glistening, the heart lying free in the sac. In most cases the latter appeared soft and large. A spotty, surface congestion was often noticed. In many of the hearts small, whitish, linear and round spots were seen on the surface, while in D 3 a large, typical scar was observed, situated on the anterior surface of the left ventricle about half-way between the apex and the junction of the anterior coronary and its anterior descending branch. The myocardium (in 5 out of 6) felt soft and somewhat flabby. In several cases different papillary muscles appeared weak and infarcted.

Lungs.—The lungs appeared congested in the lower lobes. Most lobes felt soft and spongy and on the cut surfaces oozed frothy, blood-tinged fluid. In most cases many cubic centimetres of fluid were found in the pleural cavity.

Microscopic examination.

1. Sections of lung showed definite evidence of congestion and œdema.

2. Liver—chronic passive congestion.

3. Spleen—Malphigian corpuscles somewhat enlarged; large deposits of hæmosiderin.

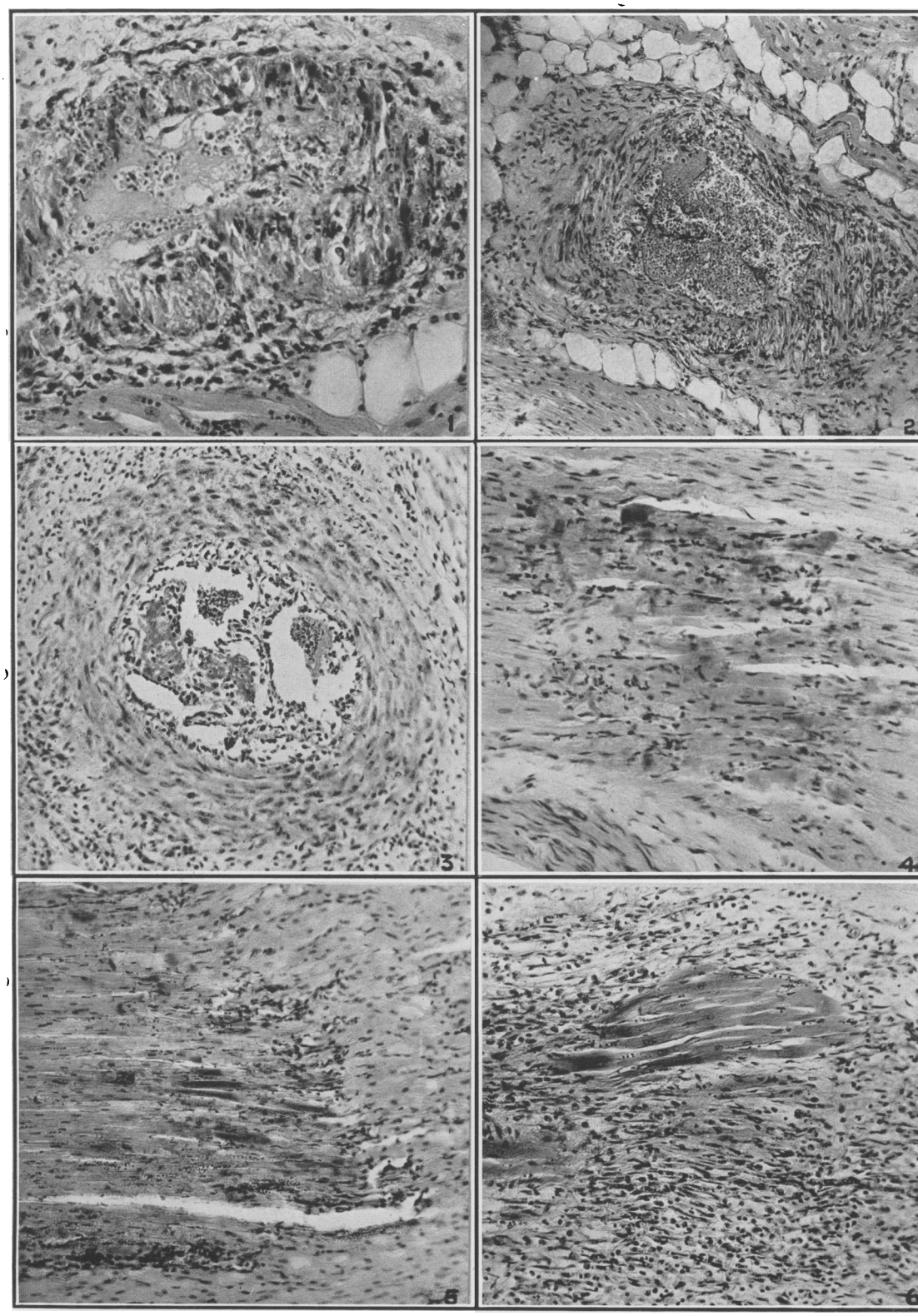

Fig. 1.—Hyaline degeneration of media, with thrombosis of artery. **Fig. 2.**—Thrombosis of coronary artery with beginning organization. **Fig. 3.**—Recanalization of thrombosed coronary artery. **Fig. 4.**—Patch of hyaline degeneration of myocardium. **Fig. 5.**—Large infarcted area with beginning fibrosis. **Fig. 6.**—Large area of fibrosis—typical scar.

4. Kidney—in D 3, very definite evidence of infarction with some fibrosis; rather severe arteriolar sclerosis and thrombosis. In the other cases little or no degeneration of the vessel walls was found.

5. The mesenteric vessels in D 3 were also thrombosed, with degeneration of media and intima.

6. Adrenal—cortex and medulla well defined; no changes noted.

7. Pancreas—no definite changes noted.

8. Intestines. — Sections taken through the more intensely congested areas showed mucosal congestion with small hæmorrhagic areas scattered throughout. Blood cells were found lying between the villi. Whether the melæna, as mentioned before, was due to rhexis or a diapedesis could not be definitely determined. In any event, areas were found where the villi were flattened and desquamation of the lining cells had taken place. No emphasis could be placed on the absence of much of the lining epithelium. All the above sections were stained with hæmatoxylin-eosin.

9. Heart.—Sections of heart were taken routinely according to the following outline: (*a*) cross-section through anterior coronary with the left descending branch, including cross-section of right and left ventricles; (*b*) flat section of right ventricle; (*c*) flat section of left ventricle — near apex; (*d*) flat section of papillary muscle — right and left; (*e*) cross-section of papillary muscle — right and left; (*f*) cross-section through septum; (*g*) cross-section across posterior coronary artery; (*h*) through S.A. node.

Examination of the above sections of the heart showed:

(*a*) *In old dogs:*

(i) Areas of hyalinization with developing fibrosis; (ii) hyaline degeneration of the media of medium and smaller sized arteries with fibrosis (Fig. 1); (iii) recent infarcts of myocardium, including papillary muscles; (iv) thrombosis of many branches of coronary artery. (Fig. 2); (v) recanalization of occluding thrombi (Fig. 3); (vi) fatty degeneration of myocardium about infarcted areas; (vii) large areas of fibrosis (Fig. 6).

(b) *In young dogs:*

(i) No arterial changes; (ii) mild to severe hyaline degeneration of myocardium (Fig. 4); (iii) a few scattered hæmorrhagic areas with cellular response; (iv) very recent infarcts of papillary muscle (D 6) (Fig. 5).

In two young dogs (D 5 and D 9) no pathological changes in the heart were noted.

Arterial damage and thrombosis were seen in the liver, intestine, mesentery, and, with infarction, in the spleen and kidney of only one animal (D 3). We were surprised to find no damage in any of the lung sections, even though the concentration of acetylcholine was, naturally, greater there than elsewhere.

DISCUSSION

The coronary vessels are believed to have a very extensive nerve supply. Woollard (1926) showed that the larger coronary vessels are supplied by both vagal and sympathetic fibres, but that this dual innervation does not extend to the medium or small-sized vessels, which are supplied solely by the vagus. The fact, as reported by Michaelow (1908), that there is such an extensive distribution of nerve fibres in the arterial walls, particularly in the medial coat, is of importance in considering our results. He describes an outer plexus in the adventitia and a deeper plexus lying between media and adventitia. Fibres from this plexus extend into the media proper where the deepest and most extensive plexus lies. This "fibre complex" of the media has been described as a "closed plexus encircling the vessel". The abundant nerve fibres in the tunica media end on the muscle fibres as small knob-like enlargements (Glaser, 1924), and penetrate the individual cells to terminate as fine, delicate mesh-like structures within the cytoplasm (Boeke, 1933). It is not yet agreed that nerve fibres exist in the intimal layer (Kuntz, 1934).

Many investigators have shown by various means that vasoconstriction of the coronary arteries occurs as a result of electrical stimulation of the vagus. Assuming that vagus stimulation is effective partly through the medium of liberated acetylcholine, and mindful of the work of Woollard (1926), Glaser (1924) and Boeke (1933), already mentioned, one could consider that any permanent changes which might be produced by prolonged excitation would be produced in the areas most abundantly supplied by vagus fibres, namely, the medial coat

of the medium and smaller-sized coronary arteries and the actual muscle fibres of myocardium (Banting, Ettinger and Hall, 1935). According to the current theory, the effect on the heart of vagus stimulation is due to the direct action on the cardiac fibres of acetylcholine discharged by the post-ganglionic fibres. Since the work of Dikshit (1934), Feldberg and Gaddum (1934), and Barsoum, Gaddum and Khayyal (1934), it is now recognized that acetylcholine is liberated at nerve synapses. Armstrong (1935), using non-innervated Fundulus hearts, suggested that the vagomimetic substance recovered from heart muscle following vagus stimulation was released only at the synapses, and produced its effect only by stimulating directly the post-ganglionic fibres.

Acetylcholine, in the amounts injected in our experiments, is rapidly hydrolyzed in the blood by its esterase as it passes from the leg-veins to the heart. The concentration of the acetylcholine in the heart or arterial blood at any one time, although small, was sufficient to produce flushing, a fall in blood-pressure, tachycardia, excessive salivation, increased peristalsis with borborygmus and diarrhœa, and an effect upon the gastro-intestinal vessels sufficient to cause hæmatemesis and melæna. These effects are not due to an alteration in the esterase system, for repeated injections of acetylcholine do not alter the activity or concentration of esterase in the blood of the dog (Lucas, Hall and Ettinger, 1935).

Clinical evidence of progressive myocardial failure with subsequent death was noted in every animal, and pathological examination showed permanent damage to the heart in all but two animals (young). This is in accord with the accepted view that the heart in myocardial failure does not always show pathological changes. As Clawson (1924) states: "Approximately half of the cases of myocardial failure show no anatomical changes in the heart muscle. The anatomical changes in the heart muscle are seldom sufficient in themselves to cause death". Paul White (1934) endorses such statements.

In those animals which at autopsy showed permanent damage to the heart, which might represent a toxic effect, there was, in the coronary arteries a hyaline degeneration of the tunica media and a tendency to thrombosis. In the myocardium there was patchy hyaline degeneration or infarction followed by extensive hyaline degeneration, with a fibrotic response.

One of the interesting observations made was that in the group of four young dogs severe gastro-intestinal symptoms developed — retching, vomiting, diarrhœa and melæna. Hæmatemesis was also observed in two of the younger dogs. At autopsy very severe congestion of the mucosa of stomach and duodenum, with blood-stained contents, was found.

In the group of old dogs no diarrhœa, no melæna or hæmatemesis was produced, and at autopsy very little congestion of the mucosa was evident.

This brings up the question of the susceptibility of the gastro-intestinal tract to parasympathetic hyperactivity and its related symptom-complex in adults, younger than those who are susceptible to the cardiac symptom-complex, as evidenced in the group of older dogs who developed more severe and earlier cardiac symptoms but failed to develop gastro-intestinal changes.

SUMMARY

Clinical degradation, with severe myocardial and coronary artery damage, was produced by the repeated injections of an abundance of a substance formed in the body and believed to be necessary for autonomic activity, and the damage was found in those areas most richly supplied by vagal post-ganglionic fibres.

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