Summary
1. The effect of cold on vagal action at the heart was studied in sheep, dogs and an isolated guinea pig atrial preparation.
   2. During cardiac output measurements in unanaesthetized sheep, by the thermodilution method, bradycardia was evoked on injection of cold indicator in eight of 12 sheep studied. This bradycardia was consistently evoked when blood pressure was increased, but not at normal blood pressure levels.
   3. In the guinea pig atrial preparation, which has one vagus nerve attached, bradycardia was evoked by electrical stimulation of the vagus nerve. When the preparation was cooled this bradycardia was potentiated.
   4. In anaesthetized dogs, the cut peripheral end of one vagus was stimulated electrically at different frequencies. The linear relationship between pulse interval and vagal frequency was then compared at deep body temperatures of 35, 37, 39 and 41°C. This comparison showed that the vagus prolonged pulse interval more effectively when the animal was cool (35°C) than when it was warm (41°C).

Key words: cardiac vagus, cold, potentiation.

Introduction
Several effects of changes in temperature on the heart and its function are well known. Decreasing the temperature will decrease the spontaneous atrial rate and increase the force of contraction [e.g. 1-6]. The durations of the action potential and of contraction are increased, and diastolic depolarization is decreased, as is the rate of rise of the action potential [5, 7].

Transient slowing of the heart rate in the cold, during cardiac output measurements by thermodilution techniques, has also been observed and this has been shown to be temperature dependent. The effect has been attributed to a direct action of cold on the pacemaker cells [8, 9].

The phenomena described in the present paper were first observed by chance in two independent studies. In the first, effects were seen during cardiac output measurements made in sheep by thermodilution techniques and the results were similar to those mentioned above. The second observation was made in isolated atrial preparations when vagal action on the heart was studied during cooling. These observations are reported here, together with results of a systematic study carried out in intact anaesthetized dogs on the actions of cold on cardiac vagal action.

Methods
Cardiac output measurements: sheep

Cardiac output measurements, by the thermodilution technique, were carried out on 12 chronically catheterized, unanaesthetized sheep in the course of another series of experiments. Bolus, intravenous injections of 10 ml of saline (0.15 mol of NaCl/l) at room temperature (20-22°C), or cold (4°C), were injected into the right atrium and the thermodilution curves were monitored. Systemic blood pressure was recorded from a femoral arterial cannula connected to a pressure
transducer (Bell and Howell: 4-327-1) and a Devices M19 pen recorder. Cardiac output measurements were carried out at resting pressure or at the peak of a pressor response. The pressor response was evoked by intravenous infusion of phenylephrine (12-600 μg/min; Neosynephrine, Winthrop), vasopressin (1-2 units/min; Pitressin, Parke Davis) or a bolus injection of angiotensin II (5 μg; Hypertensin, Ciba). In four sheep sympathetic β-adrenergic effects were blocked by propranolol (15 mg bolus followed by 0.5 mg/min infusion; Inderal, ICI; [11]). Deep body temperature was measured to the nearest 0.1°C with a temperature probe closed within the peritoneal cavity (Bosch thermometer 15.22). The animal was heated with lamps and cooled with ice packs to enable cardiac vagal action to be studied at temperatures of 35, 37, 39 and 41°C. The animal was stabilized under deep surgical anaesthesia at 35°C when this temperature was reached, and thereafter the range of temperatures under study was covered within 1 h. At each temperature the vagus was stimulated supramaximally (usually, 1 ms and ~10-20 V) at various frequencies and the relationship between vagal frequency and pulse interval plotted for each temperature.

No results are included from series in which it was necessary to supplement the anaesthesia within the period of temperature variation.

Results

Sheep

It was observed by chance, in the course of other experiments on unanaesthetized sheep, that on some occasions when cardiac output was measured by the thermodilution technique, the injection of 10 ml of saline (0.15 mol of NaCl/l) (20-22°C) into the right atrium caused a pronounced fall in heart rate. This effect was transitory and heart rate returned to pre-injection levels within a few beats. The effect was seen in five of the nine animals given injections of saline at this temperature. Furthermore, in these five animals the intra-atrial injections of cold saline had no effect on heart rate until after arterial pressure had been raised by a pressor agent. The pressor agents used in the study were phenylephrine, angiotensin and vasopressin.

Injections of colder saline (10 ml at 4°C) were used in another three sheep. In all of these the injections evoked marked bradycardia, but again only after arterial pressure had been raised by a pressor agent. The effect was seen in eight of the 12 animals studied (four of these eight had sympathetic β-adrenergic effects blocked by propranolol), and in these it appeared most reliably only after the blood pressure had been raised by 20-40 mmHg by infusions of pressor agents. In these circumstances it can be assumed that vagal tone would have been enhanced through the arterial baroreceptor reflex, which would account also
Cold potentiates vagus

Guinea pig atria

The second chance observation reported here was made during studies on vagal action in the guinea pig atrial preparation.

Here intermittent, brief electrical stimulation of the vagus evoked a prompt, marked and reproducible bradycardia. When the Krebs' solution in the organ bath at 34 ± 1°C was cooled by flushing the bath with Krebs' solution at a temperature closer to room temperature, several effects of cold were reliably seen. There was a spontaneous decrease in the control rate of beating and an increase in contractility. In addition to these well-known effects of cold on the heart the decrease in heart rate evoked by vagal stimulation was always markedly potentiated when the preparation was thus cooled. These effects of cold are illustrated in Fig. 2. In the cold (approx. 30-33°C) the fall in heart rate induced by vagal stimulation was greater by at least 50% than the fall in the control rate of beating in all ten guinea pig preparations studied here.

Dogs

As in the guinea pig preparation, stimulation of the peripheral end of the vagus in the anaesthetized dog evokes a prompt and marked bradycardia, i.e. as the frequency of vagal stimulation is increased the pulse interval increases. This relationship between frequency of vagal stimulation and pulse interval is linear and the slope of the relation between the two can define the effectiveness of the vagus in slowing the heart [12]. In the present study the linear relation between pulse interval and vagal frequency was obtained at the temperatures 35, 37, 39 and 41°C. In all six animals the vagus became more effective with cooling at prolonging the pulse interval.

An example of this, from one dog, is illustrated in Fig. 3(a), where linear regression lines are fitted to the set of data points obtained at each temperature studied. It can be seen that the slope of this line is greatest at 35°C and least at 41°C, indicating that the vagus is more effective in slowing the heart at cooler temperatures. The effect of cold on the pulse interval, in the absence of vagal stimulation, is small in comparison with its effects during such stimulation. These points are clear also in Fig. 3(b), where the same data are plotted as pulse interval against temperature, for various frequencies of vagal stimulation.

The linear regression lines drawn similarly for all six dogs at the four temperatures are shown in
Discussion

The work described here includes and elaborates upon the chance findings that cardiac vagal action is potentiated in the cold. This potentiation of vagal action causes much larger increase in pulse interval than the direct action of cold on the cardiac pacemaker; this is evident from the studies in the dogs and in the guinea pig atrial preparations here (e.g. Figs. 2 and 3). Cold has been shown to reduce the maximum frequency of transmission in vagal nerves [13], and could conceivably have reduced the apparent size of the effect reported here. Of particular relevance to physiologists and clinicians is our finding that bradycardia is especially likely to be seen in response to bolus injections of cold thermodilution indicators when vagal tone is high (Fig. 1). We gained the impression, without systematically studying it, that this effect was most reliably seen when vasopressin was used to raise blood pressure and least so when angiotensin was used to raise it; this would fit with our previous observations that vasopressin increased efferent vagal activity [14], while angiotensin inhibits it [15]. Our present findings make the bradycardias previously reported in some human subjects [8] more likely to be due to an action of cold upon a vagal mechanism, than directly upon intrinsic pacemaker activity. Certainly, there is a threat to the reliability of cardiac output measurements made in such conditions. It may be that apprehension, cardiovascular disease, or other factors, are responsible for there being little or no resting vagal tone in most subjects in the conditions in which cardiac output is most often determined by thermodilution. If so, the phenomenon we describe should not be a problem.

The mechanism of action of cold in potentiating vagal action was not studied here. There are several possibilities. A decrease in the activity of acetylcholinesterase by cold could account for the increased action of the vagus: acetylcholinesterase activity is reduced in cold [16]. An increase in acetylcholine release could also account for the results described here. No measure of acetylcholine release has been made at the sinus node or at the parasympathetic ganglion in the heart. At the neuromuscular junction, however, acetylcholine release is increased with increasing temperature [16], which would not fit our observations here. A change in the sensitivity of the pacemaker could also occur in response to cold. Possibly, the lifetime of channels opened by vagally liberated acetylcholine could be prolonged in the cold, leading to enhancement of the acetylcholine effect [17]. Certainly, we can say...
with confidence that the site of action of cold is localized to the peripheral end of the vagus from the results of the experiments on dogs and in the guinea pig atria reported here. However, as the parasympathetic ganglion lies close to the sinus node, pre- and post-ganglionic sites must be considered.

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References