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# STUDIES ON THE COMPARATIVE ACTIONS OF CARBAMYLCHOLINE, PHYSOSTIGMINE AND NEOSTIGMINE IN DIFFERENT SPECIES OF DOMESTIC ANIMALS.

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## INTRODUCTION.

Although carbamylcholine, physostigmine and neostigmine are all classed as parasympathomimetics, their actions are not identical. Carbamylcholine acts by direct stimulation of the cholinergic effector organs, whereas physostigmine and neostigmine inhibit the enzyme cholinesterase thereby prolonging and intensifying parasympathetic activity. Furthermore, these substances exhibit differences in their intensity of action on various organs and organ systems.

For instance, in comparing the actions of neostigmine and physostigmine, Grollman and Slaughter (1947) state that neostigmine is as active as physostigmine in promoting intestinal peristalsis but has less action on the heart and circulation. From considerable personal experience, the authors can state that the action of neostigmine differs from that of carbachol in several respects, the most obvious being the absence of profuse salivation, bronchial secretion (coughing) and vasodilation (cardio-acceleration). Furthermore, neostigmine has been found to be a more reliable purgative in horses than carbamylcholine.

In view of these differences, it was decided to conduct a systematic study of the pharmacological actions of these three drugs in various species of domestic animals.

## EFFECTS ON THE HEART AND BLOOD VASCULAR SYSTEM.

Several dogs, one donkey and one goat were used. The animals were anaesthetised, pentobarbital sodium being used on the dogs and chloral hydrate on the goat and donkey. The arterial blood pressure was recorded by direct canulisation to a mercury manometer. The drugs were injected intravenously at a standard dosage of 1 mg. per 100 lb. live weight in the case of carbachol and 2·5 mg. per 100 lb. in respect of both physostigmine and neostigmine. The relative tracings will be found in figures 1 to 5 at the end of this article.

### *Results.*

Carbamylcholine chloride caused a profound and immediate drop in blood pressure in all the dogs. (Tracings 1, 2 and 3.) In the donkey it caused the diastolic pressure to drop to 0, while the systolic pressure rose from ± 100 to over 150 (tracing 4). This enormous increase in the pulse pressure and the complete collapse of diastolic pressure can only be ascribed to acute vaso-collapse.

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In the case of the goat, carbamylcholine caused an intermittent complete inhibition of the heart (see tracing 5) occurring over the first six minutes, followed by a depression of the mean arterial pressure and an increase in the pulse pressure.

Neostigmine was shown to cause an increase in mean arterial pressure in the dog and also in the donkey (see figures 3 and 4). Physostigmine also caused a slight rise in the blood pressure of the dog (tracing 2).

*Discussion.*

Clinical observations on animals treated with therapeutic doses of carbachol invariably reveal an acceleration of the heart and the development of a water-hammer pulse. This indicates that, in moderate doses, this drug causes a fall in blood pressure through vasodilation with consequent reflex cardiac acceleration. The presence of vaso-collapse was confirmed in the blood pressure tracing made from the donkey by the severe fall in diastolic pressure indicating a great reduction in the peripheral resistance.

In larger doses carbachol causes cardiac inhibition or even arrest due to its direct action on the heart.

With regard to physostigmine Grollman and Slaughter state— *"The changes in the circulation require further investigation. Small doses slow the pulse and increase the blood pressure, while larger doses are followed by greater slowing of the heart and a fall in blood pressure"*. They also state— *"The increased blood pressure has been the subject of some discussion. It seems independent, in part at least, of the vasomotor centre, for it is not prevented by section of the spinal cord or of the splanchnic nerves"*.

The present authors considered that the rise in blood pressure might be due to the release of adrenaline caused by cholinergic stimulation of the nerve endings in the adrenal medulla, although it is admitted that this is not in accord with the statement quoted above viz. that it is not prevented by section of the splanchnic nerves. Confirmation of the adrenaline theory was obtained by the following experiment, however.

An isolated rabbit's heart was perfused in the usual manner with tyrode solution containing 1% defibrinated rabbit's blood. When the heart had attained a steady beat at normal body temperature, 1 ml. of a second rabbit's blood was defibrinated, diluted with 4 ml. warm tyrode and injected into the perfusion fluid as it entered the heart. As seen in tracing 6 this had no effect. The second rabbit was then injected with ·125 mg. neostigmine and blood was again withdrawn two minutes later. Injection of this blood caused a marked augmentation of the heart beat typical of small doses of adrenaline.

When the beat had returned to normal a third rabbit was injected with ·05 mg. carbamylcholine and bled two minutes later. Injection of 1 ml. of this blood caused profound inhibition of the heart. That this was not due to normal deterioration of the preparation is shown by its recovery recorded 30 minutes later.

This experiment therefore proved that the blood of the rabbit injected with neostigmine contained a cardiac stimulant, presumably adrenaline, while that of the rabbit which received carbachol had a cardio-inhibitory action, presumably due to circulating carbachol. This latter aspect indicates the tremendously powerful cardio-inhibitory action of this drug. The rabbit was originally injected at a rate of 1 mg. per 100 lb. live weight and 1 ml. of its blood contained sufficient carbamylcholine to cause severe cardiac inhibition.

It may therefore be postulated that the rise in blood pressure after small doses of neostigmine or physostigmine is due to vasoconstriction caused by adrenaline secretion. The simultaneous slowing of the heart might then be ascribed to the normal depressor reflexes. In larger doses these drugs inhibit the heart thus causing a fall in blood pressure.

From clinical observations, adequate purgative doses have no detectable effect on the pulse rate or character.

## Effect on the Lungs.

No recordings were made of the effects on the bronchial musculature or glands but clinical observations showed that carbamylcholine causes copious bronchial secretion as evinced by marked rahls and coughing even after small doses. Neostigmine and physostigmine do not cause these symptoms.

## The Alimentary Tract.

*Salivary Secretion.*

Throughout the experiments copious salivation after carbachol administration was noted in all species. This did not occur after neostigmine or physostigmine. So severe was the salivation induced by carbachol that the anaesthetised animals had to be tubed to prevent suffocation. Figures with regard to salivary flow were obtained from two goats. The animals were anaesthetised with chloral hydrate and the parotid ducts catheterised. The amount of saliva secreted per five minutes was then measured before and after injection of the drugs. The results are shown in graph 1.

It will be noted that carbacol caused a five- to six-fold increase in parotid secretion whereas neostigmine only increased it by some 25%. Physostigmine actually caused a decrease, which may also be ascribed to adrenaline action.

*Stomach and Large Intestine.*

*Dog.*—As will be seen from tracings 1, 2 and 3, carbachol caused spastic contraction of the stomach followed by violent rhythmic contractions. Physostigmine had no effect on the stomach while neostigmine actually caused a drop in tone.

All three drugs caused increased motility of the colon.

*Donkey* (tracings 7, 8 and 9). Carbachol caused violent motility of the stomach and had no effect on the caecum.

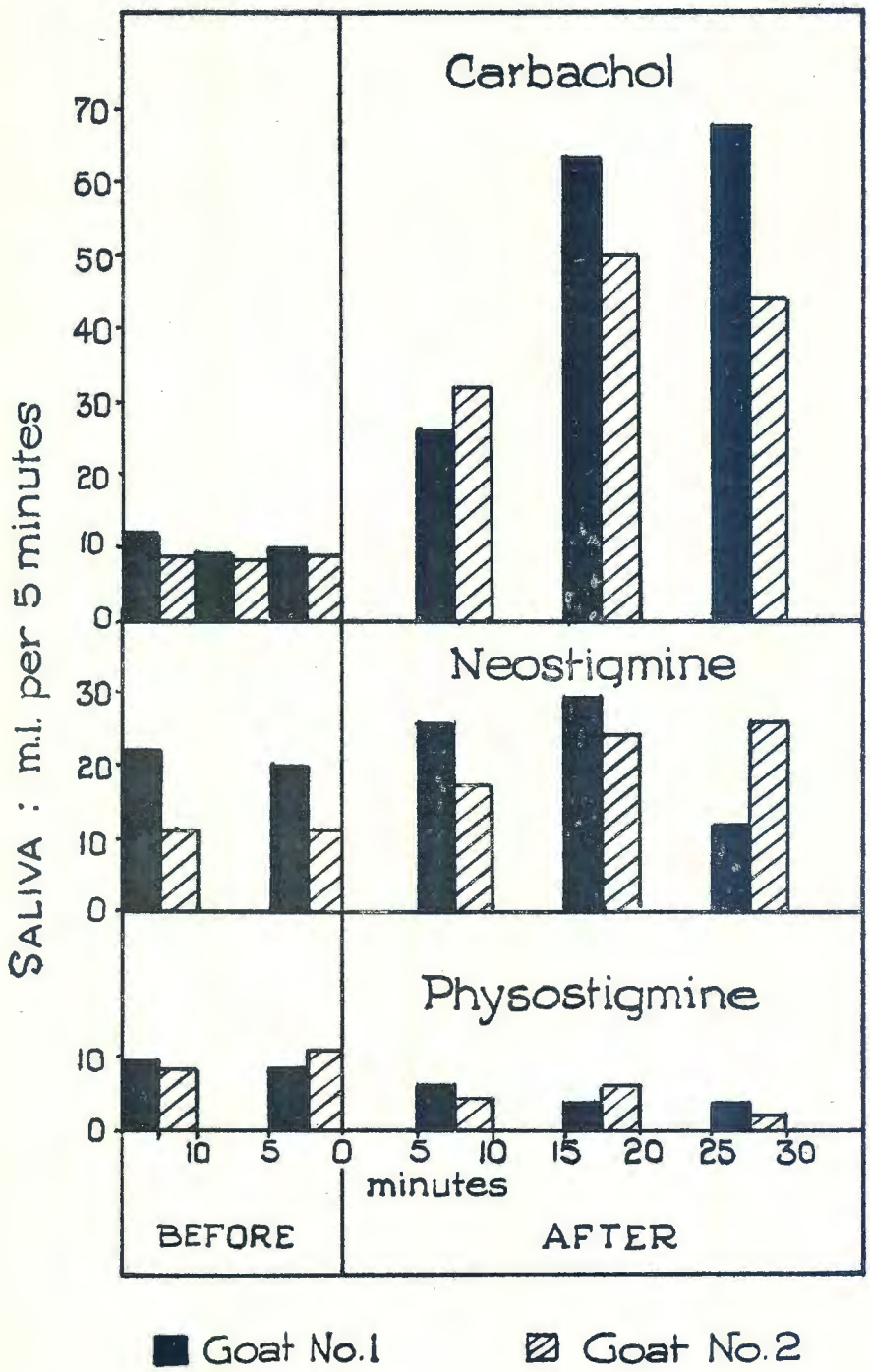
Conversely physostigmine caused marked caecal motility without affecting the stomach. Neostigmine produced hypermotility of both stomach and caecum.

*Sheep* (tracings 10, 11 and 12). Carbachol caused hypermotility with loss of co-ordination in the rumen. The mixing movements actually became weaker while the eructation contractions were increased in force but entirely functionless due to the complete inhibition of the reticulum. The force of the caecal contractions was slightly reduced. The inhibitory action on the reticulum has been reported by Duncan (1951).

Physostigmine increased the strength of the ruminal contractions without interfering with their co-ordination. The reticulum and caecum were unaffected.

GRAPH No. 1.

The Effects of Carbamylcholine, Neostigmine, and Physostigmine on the Salivary Secretion of the Goat.




Note marked increase after carbachol, slight increase after neostigmine, and sligt decrease after physostigmine. For explanation see text.

In the normal dose of 2·5 mg. per 100 lb., neostigmine had a similar action to physostigmine on the rumen but also increased the strength of the reticular contractions. With double the dose the reticular contractions were reduced in strength while the caecum was still unaffected.

It will be noted that these parasympathetic stimulants are not indicated as purgatives for the sheep, as they have little effect on the hind gut. This has been borne out by clinical experiences. Repeated doses of carbachol have to be given to cause the passage of soft faeces and in the meantime the motility of the forestomach is interfered with.

Purgation occurs in cattle with greater ease but the action on the forestomachs is identical.

### Effect on the Bladder.

In the dog (tracings 1, 2 and 3) carbachol caused marked contraction of the urinary bladder while physostigmine and neostigmine had little effect. This difference in action was also noted in the donkey (tracing 4).

### Skeletal Muscle.

Throughout the experiments it was noted that all three drugs caused muscle tremors but that this was more marked in the case of neostigmine. This is in accordance with the findings in human medicine where neostigmine is used in the treatment of *myasthenia gravis*.

The results of the above experiments can best be summarised in the form of the following table:—

| Organ. | Carbachol. | Physostigmine. | Neostigmine. |
|---|---|---|---|
| Heart | Small doses Acceleration<br>Large doses inhibition | Small doses, slight slowing<br>Large doses, marked slowing | Small doses, slight slowing.<br>Large doses, marked slowing. |
| Vasotone | Marked fall | Rise | Rise. |
| Bl. press | Marked fall | Slight rise | Slight rise. |
| Bronchi | Secretion and constriction | No effect | No effect. |
| Salivary glands | Marked stimulation | Slight decrease | Slight increase. |
| Stomach— | | | |
| Dog | Violent contraction | No effect | No effect. |
| Donkey | Violent contraction | No effect | Contraction. |
| Rumen | Violent contraction | Increased force | Increased force. |
| Reticulum | Inhibition | No effect | Increased force. |
| Colon— | | | |
| Dog | Contraction | Contraction | Contraction. |
| Caecum— | | | |
| Donkey | No effect | Contraction | Contraction. |
| Sheep | No effect | No effect | No effect. |
| Bladder— | | | |
| Dog | Contraction | Slight contraction | Slight contraction. |
| Donkey | Contraction | — | No effect. |

Note.—Except where otherwise stated effects of normal doses given.

## CONCLUSIONS.

The experiments described emphasise the extreme danger attached to the use of carbamylcholine, especially in debilitated and anaemic animals. Doses insufficient to cause purgation result in a profound drop in the diastolic blood pressure due to peripheral vasodilation accompanied by reflex cardio-acceleration. As the coronary blood flow depends on the diastolic pressure and duration, this results in a reduced blood supply to the heart. Larger doses aggravate the fall in blood pressure by inhibiting the heart itself. Furthermore, the copious bronchial secretion, together with constriction, causes marked respiratory embarrassment. Physostigmine and neostigmine, in therapeutic doses, have none of these undesirable effects.

The fact that carbachol causes inco-ordinated contractions of the rumen and inhibits the reticulum makes it entirely unsuitable as a ruminotorric. The inhibition of the reticulum contraindicates its use in bloat as the motility of this organ is essential to the eructation of gas.

The only indication for the use of carbachol is its superior action on the urinary bladder. It also has a greater action in producing contraction of the simple stomach and salivary secretion, but these effects are seldom required.

As regards the comparison of physostigmine and neostigmine it will be noted that these drugs had very similar actions. However, the impression was gained that neostigmine has certain advantages. Horses treated with neostigmine showed satisfactory purgation with less signs of colic and distress than those treated with physostigmine.

## SUMMARY.

In a series of experiments the comparative actions of carbamylcholine, physostigmine and neostigmine were compared in different species.

Carbamylcholine was shown to have the following undesirable effects:—

(i) Respiratory distress due to bronchial secretion and constriction.
(ii) Marked drop in blood pressure due to vasocollapse.
(iii) Production of inco-ordinated contractions of the rumen and inhibition of the reticulum.
(iv) Profuse salivation.
(v) Little effect on the large intestine of the horse or sheep.

It was more active on the stomach and bladder than the other two drugs tested.

Physostigmine and neostigmine had very similar actions characterised by:—

(i) A slight rise in blood pressure after therapeutic doses. Evidence is produced indicating that this may be due to adrenaline secretion.
(ii) Very little effect on the heart rate, salivary secretion or bronchioles.
(iii) Little effect on the stomach of the dog or donkey. Increased strength of the contractions of the rumen and reticulum. Marked stimulation of the large intestine in all species.

The impression was gained that neostigmine produced purgation in equines and dogs with less signs of colic than did physostigmine.

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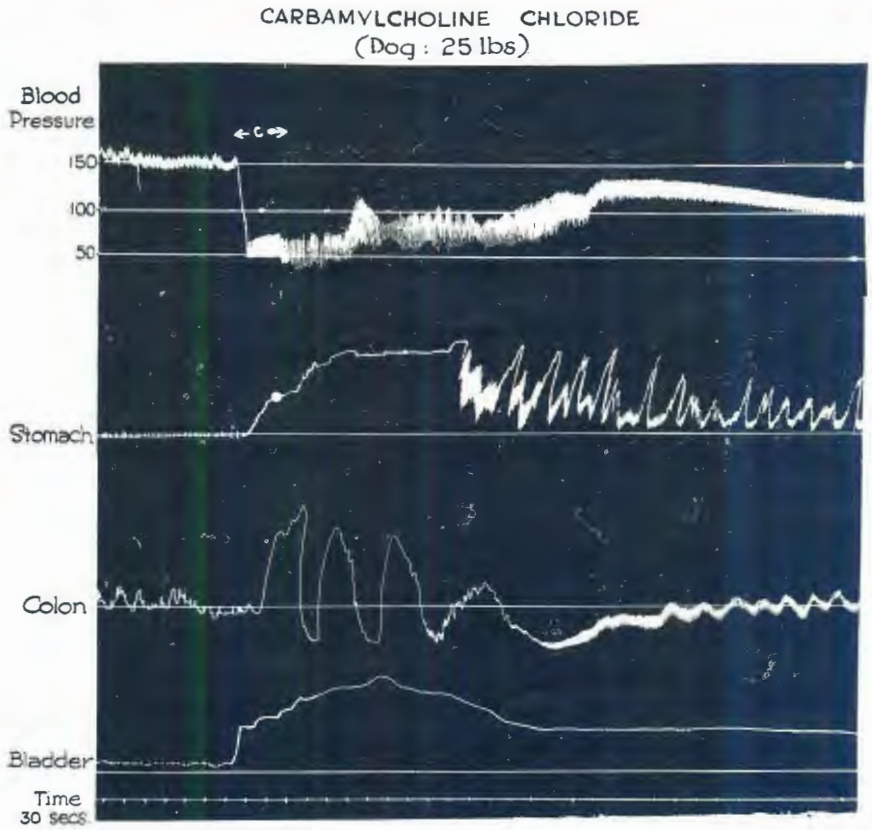
They also wish to acknowledge the generous supplies of prostigmin (neostigmine—Roche) made available by S.A.R.P. (Pty.) Ltd.

REFERENCES.

DUNCAN, DOROTHY L. (1951). Effect of some choline esters upon the rumen and reticulum of sheep. *Jl. Physiol.*, Vol. 115, p. 75.

GROLLMAN, A. AND SLAUGHTER, D. (1947). Cushney's pharmacology and therapeutics (13th edit.). J. and A. Churchill Ltd., London.

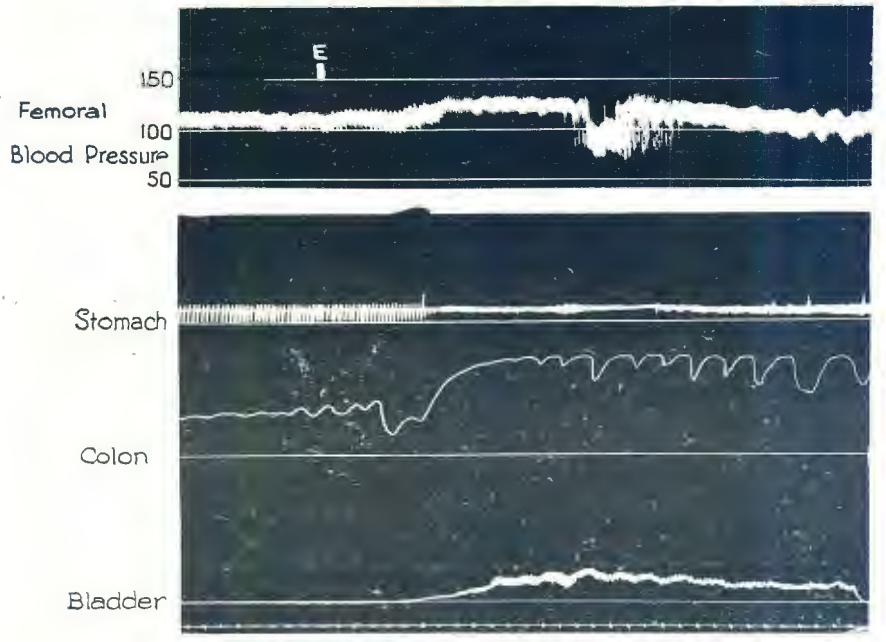
TRACING No. 1.




←C→ = ·125 mg. carbachol intravenously.

TRACING No. 2.

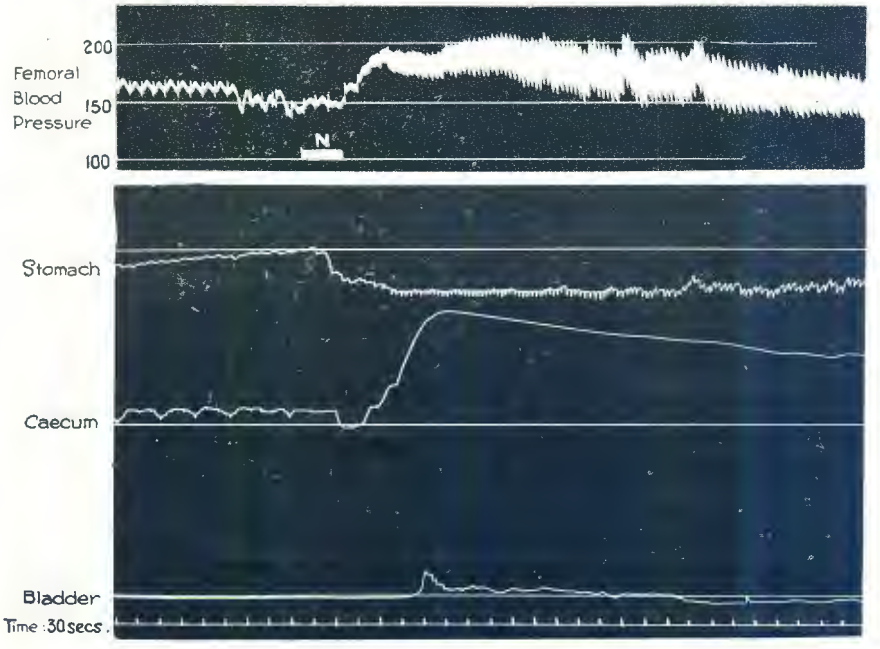
Physostigmine (Dog: 78 lb.).




E = Injection of Physostigmine i.v.

TRACING No. 3.

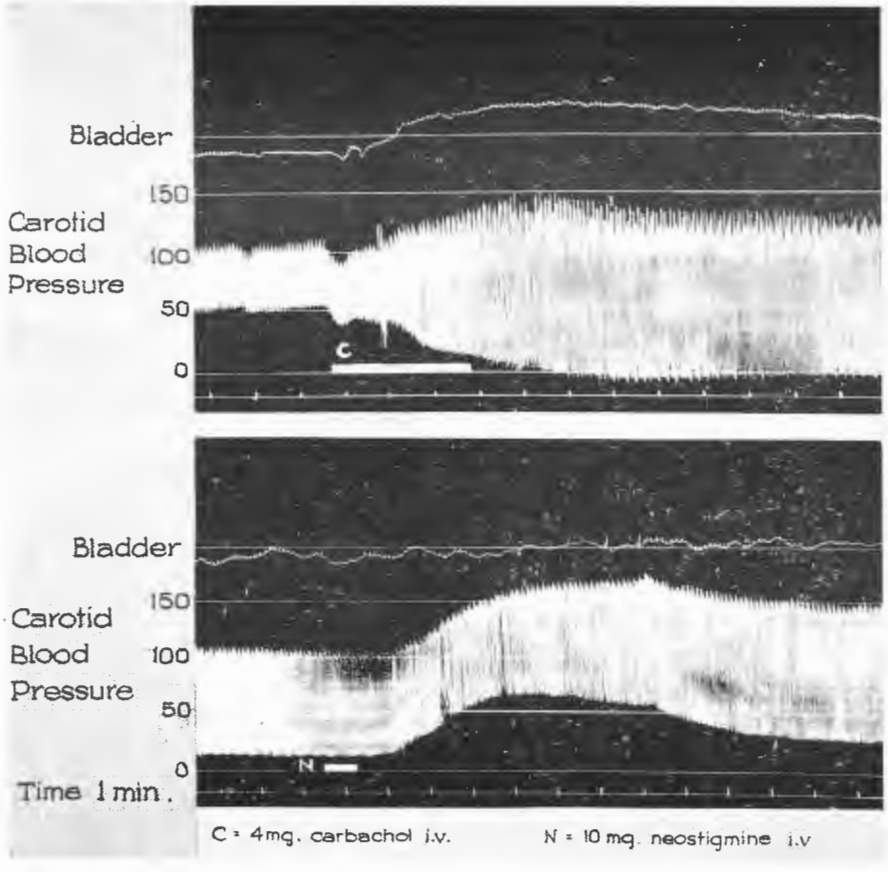
NEOSTIGMINE : DOG (40 LBS.)




N = Injection of 2 mg. Neostigmine i.v.

TRACING No. 4.

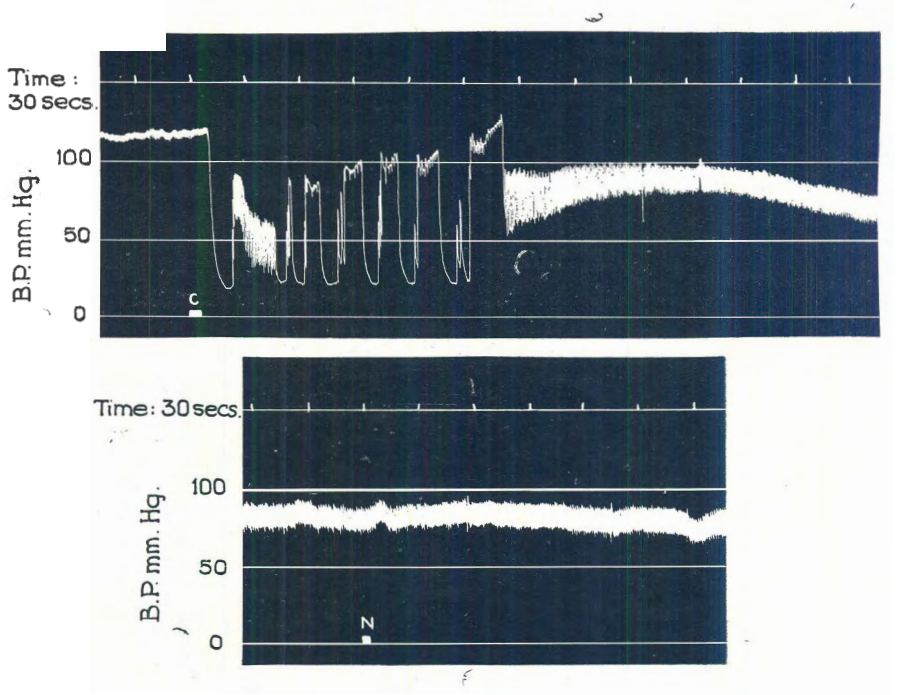
(Donkey: 400 lb.).




Upper tracing: The effect of 4 mg. carbachol i.v. injected at C.
Lower tracing: The effect of 10 mg. neostigmine i.v. injected at N.

TRACING No. 5.

ts of Carbamylcholine and Neostigmine on the Femoral Blood Pressure of the Goat (65 lb.).

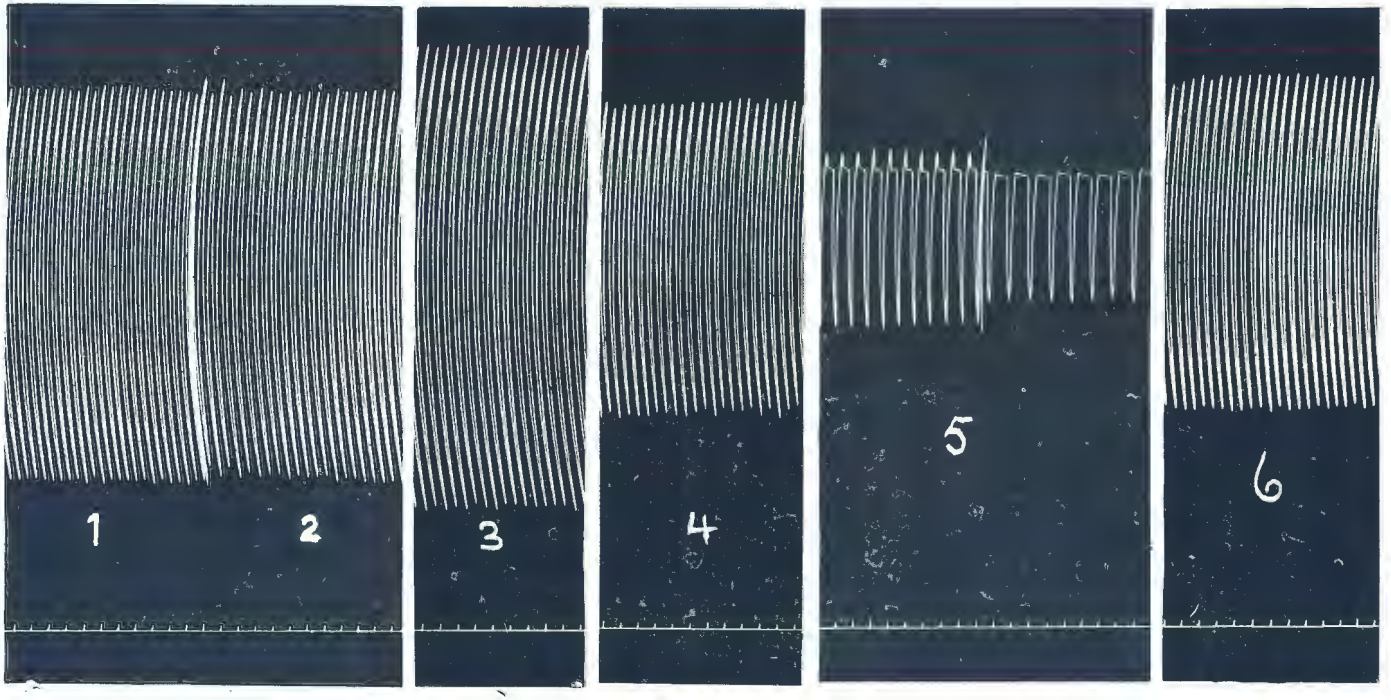



ove: C = Injection of ·6 mg. Carbamylcholine. Note intermittent complete inhibition of the heart and subsequent drop in mean blood pressure.

low: N = Injection of 2 mg. Neostigmine one hour later.

TRACING No. 6.

The Isolated Perfused Rabbit's Heart.




1 = Normal beat.

2 = No effect from addition of 1 ml. defibrinated blood from rabbit No. 2.

3 = Augmentation after the addition of 1 ml. rabbit's blood after injection of neostigmine.

4 = Normal beat regained.

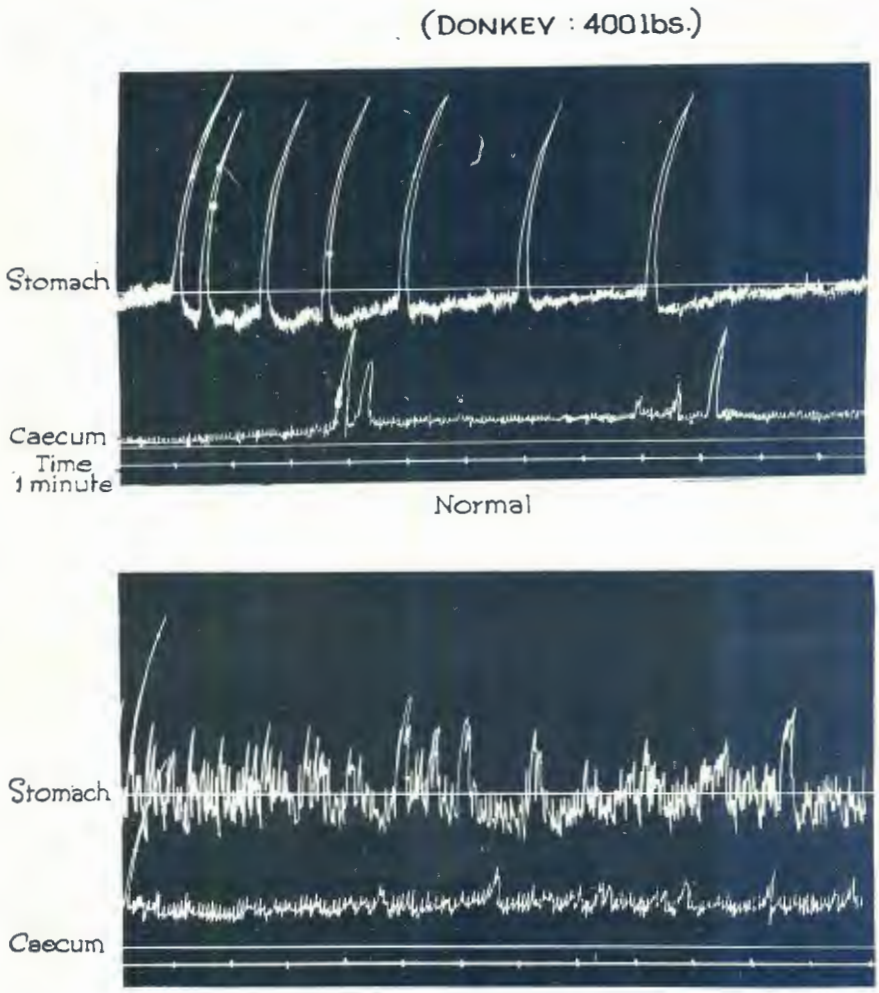
5 = Inhibition after the addition of 1 ml. rabbit's blood after injection with carbamylcholine.

6 = Normal beat regained.

For full explanation see text.

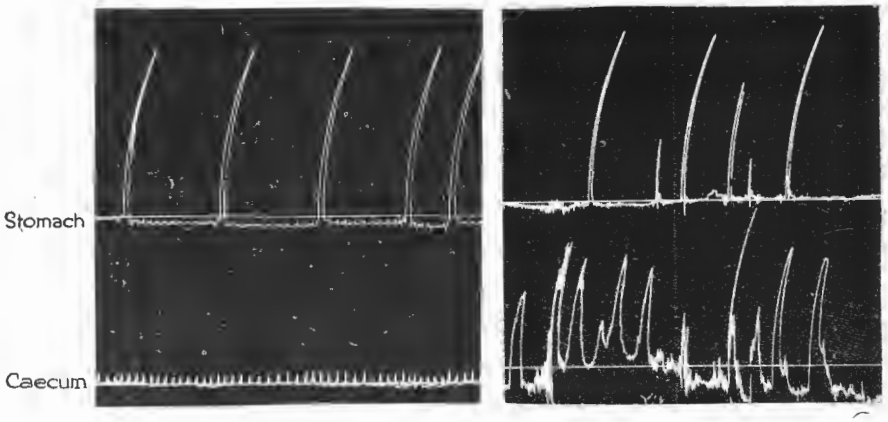
TRACING No. 7.

The effect of Carbamylcholine on the stomach and caecum of the Donkey.



TRACING No. 8.

The effect of Physostigmine on the stomach and caecum of the Donkey.

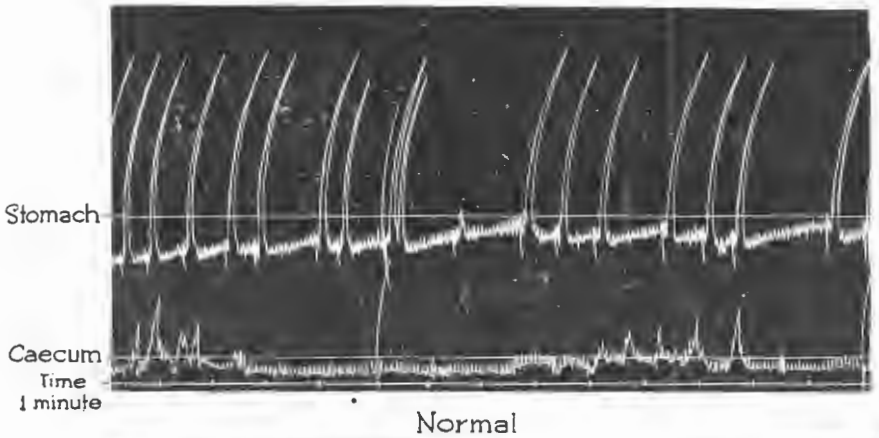



Normal.

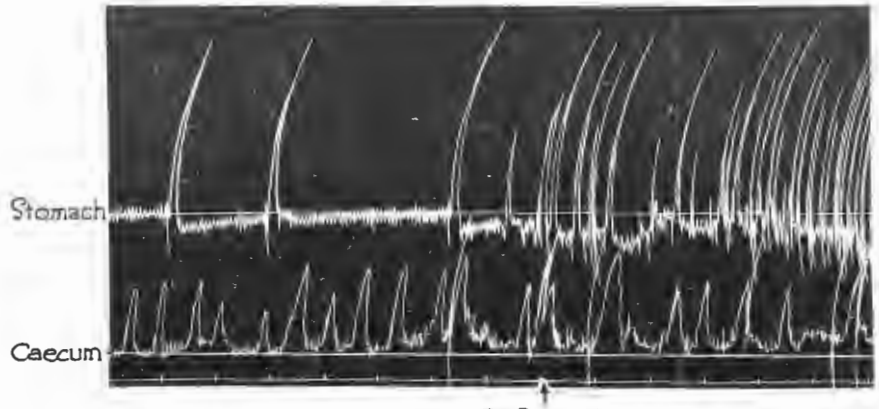
10 Minutes after the injection of 10 mg. physostigmine sub-cut.

TRACING No. 9.

The effect of Neostigmine on the stomach and caecum of the Donkey.




Normal

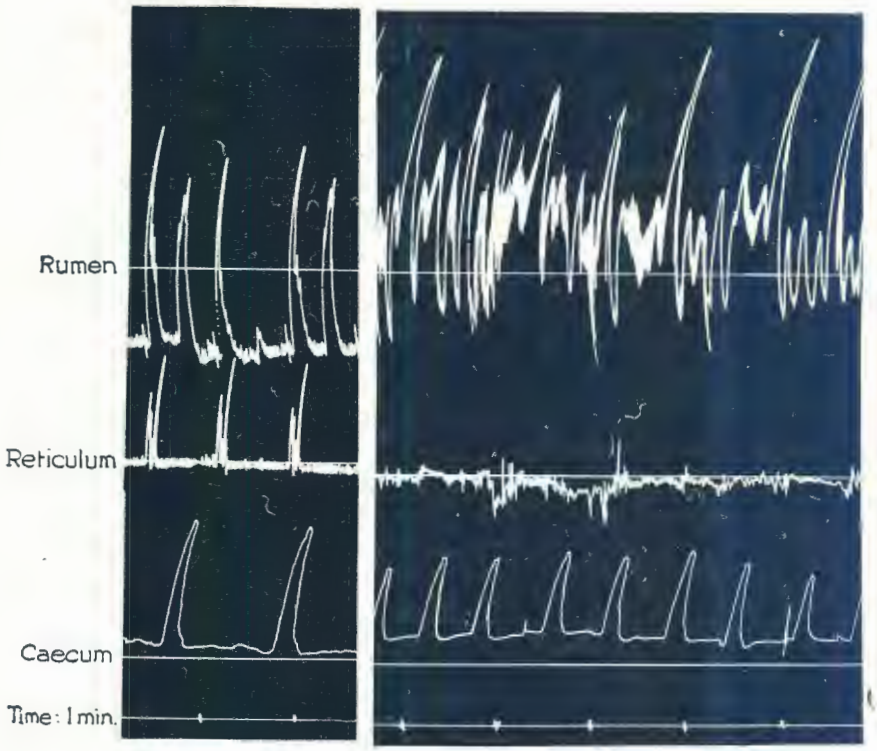



10 mins. after injection of 12·5 mg. Neostigmine sub-cutaneously.

Note hypermotility of both stomach and caecum.

TRACING No. 10.

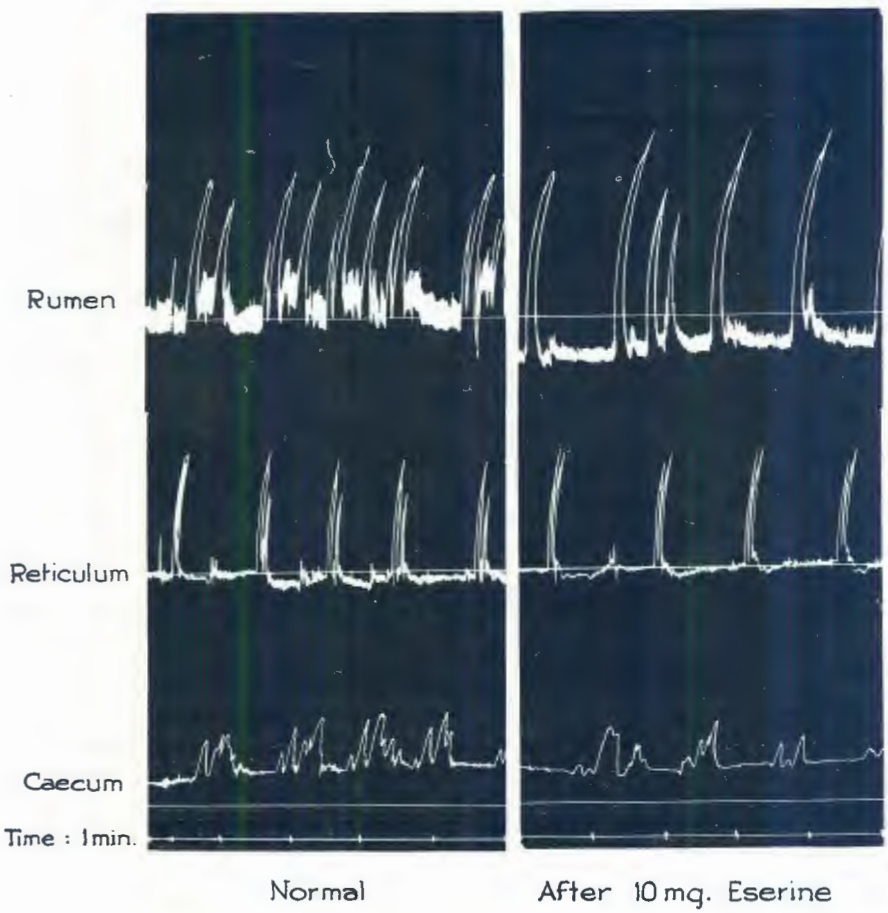
The effect of Carbamylcholine on the motility of the Alimentary Tract of the Sheep.




Note inco-ordinated spasms of the rumen and inhibition of the reticulum.

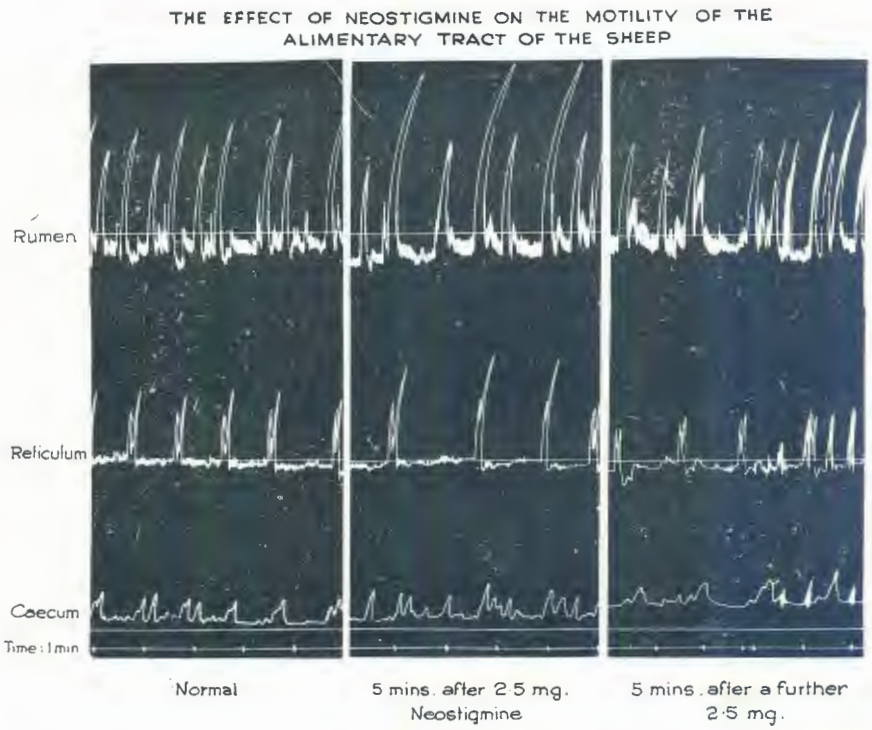
TRACING No. 11.

The effect of Physostigmine on the Motility of the Alimentary Tract of the Sheep




Note increased strength of ruminal contractions; no effect on reticulum or caecum.

TRACING No. 12.




Note normal dose increased strength of contraction of both rumen and reticulum.
Double dose decreased strength of contraction of reticulum.
Caecum unaffected throughout.