knowledge of the movement of the gut in the mammalian foetus is mainly derived from observations on small laboratory animals. yanase (1907) made a systematic study of pre-natal intestinal movement in the guinea-pig, and observed that the appearance of peristalsis coincided with the time of development of the longitudinal muscle of the gut, at about the 27th day of gestation. at the stage when circular muscle alone was present, he was able to elicit only local constriction by mechanical or faradic stimulation. foetal gastric movement was observed in the rabbit (tani, 1927) and in the cat by friedman (1934), windle & bishop (1939), and becker & windle (1941). the last-named described rhythmic and persistent gastric peristalsis in cat foetuses of 35-40 days. becker, windle, barth & schultz (1940) found that thorotrast injected into the amniotic sac of the guinea-pig circulated through the alimentary tract of the foetus from the 42nd day onwards; and human foetal swallowing is said to occur from the fifth month (windle, 1940). while there is evidence, therefore, that alimentary tract movement commences in pre-natal life, nothing is known of the factors controlling the onset of movement or of the nature of the individual movements.

the movements of the stomach of the foetal sheep are described here, as this species might be expected to yield more evidence than others concerning the spontaneous occurrence of movement, the nature of the movement, and the part played by the vagus in initiating it, for in adult ruminants the movements of the parts of the stomach are characteristic and marked. further, barcroft & barron (1939) observed swallowing and suckling movements in the foetal sheep, and as suckling in the lambs is associated with a reflex closure of the structure known as the oesophageal groove, the onset of the reflex in foetal life can be expected to provide some index of the post-natal activity.

experimental

scottish blackface and dorset horn ewes were used. tupping dates were recorded and pregnancy was confirmed radiologically. with the blackfaces radiological diagnosis of pregnancy was usually successful after 60 days, but it was less successful in the larger dorset ewes at that age. this method enabled ewes with twin lambs to be selected when required.

the supply of material was planned so that foetuses from 40 days onwards could be obtained at ages increasing by 5–10-day intervals until full term. table 1 gives the details of the stages selected.
Motor responses in the stomach of the foetal sheep

Table 1. The sheep used and the foetal ages at which the experiments were performed

<table>
<thead>
<tr>
<th>Ewe no.</th>
<th>No. of foetuses</th>
<th>Foetal age (days)</th>
<th>Ewe no.</th>
<th>No. of foetuses</th>
<th>Foetal age (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>866</td>
<td>1 (+1)*</td>
<td>40</td>
<td>694</td>
<td>2</td>
<td>c. 74</td>
</tr>
<tr>
<td>862</td>
<td>2</td>
<td>41</td>
<td>693</td>
<td>2</td>
<td>80</td>
</tr>
<tr>
<td>690</td>
<td>2</td>
<td>50</td>
<td>680</td>
<td>2</td>
<td>81</td>
</tr>
<tr>
<td>705</td>
<td>2</td>
<td>50</td>
<td>602</td>
<td>2</td>
<td>90</td>
</tr>
<tr>
<td>867</td>
<td>2 (+1)*</td>
<td>50</td>
<td>695</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>711</td>
<td>1</td>
<td>c. 54</td>
<td>706</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>861</td>
<td>2</td>
<td>55</td>
<td>700</td>
<td>1</td>
<td>110</td>
</tr>
<tr>
<td>863</td>
<td>1</td>
<td>60</td>
<td>697</td>
<td>2</td>
<td>120</td>
</tr>
<tr>
<td>708</td>
<td>2</td>
<td>60</td>
<td>865</td>
<td>1</td>
<td>120</td>
</tr>
<tr>
<td>702</td>
<td>2</td>
<td>70</td>
<td>868</td>
<td>2</td>
<td>131</td>
</tr>
<tr>
<td>704</td>
<td>2</td>
<td>70</td>
<td>710</td>
<td>1</td>
<td>140</td>
</tr>
</tbody>
</table>

* 1 foetus carried to full term.

The ewes were given urethane intravenously in 25% solution until they were unable to stand. 'Duracaine' was then injected into the subarachnoid space in the lower lumbar region. Further urethane was given as necessary.

Nos. 861, 862, 863, 866 and 867, all Dorset Horns, were anaesthetized with 'Nembutal'. Caesarean section and the experiment on the foetus were performed under aseptic conditions, and the mother was allowed to recover. As these experiments were all performed during the early stages of gestation, anaesthetic effects upon the foetus were not observed. Nos. 866 and 867 had twin and triple foetuses respectively, one of which in each ewe escaped notice, and each ewe bore at full term, without assistance, a normal, healthy lamb. Hence surgical interference with the uterus as early as the 2nd month does not necessarily lead to abortion. In the case of non-recovery experiments the ewe was placed on her back in a bath filled with warm saline, in which the foetus was immersed, on Caesarean section being performed through the mid line.

The following observations were made on the foetus:
(a) presence or absence of swallowing or suckling;
(b) presence or absence of spontaneous gastric movement;
(c) the results of vagal stimulation;
(d) histological examination of the stomach.

(a) Spontaneous swallowing was studied in only five foetuses. The head of a foetus was located, a small incision was made in the uterine wall, and about 5 ml. of Indian ink was injected into the amniotic sac. The hole in the amnion was then quickly tied off, the uterine incision was sutured, and the uterine horn returned to the abdominal cavity, in an effort to disturb the foetus as little as possible while the twin was examined.

In studying induced swallowing, an incision was made in the uterus and the mouth was brought up to it. Barium sulphate suspension was dropped into the mouth, the rounded tip of the pipette being placed on the tongue in order to stimulate suckling. For the largest foetuses a finger was placed in the mouth and gently moved on the tongue. Foetuses were radiographed immediately after the end of the experiment, i.e. about 30 to 60 min. after the barium was given.
(b) and (c) After observing swallowing the foetus was drawn from the membranes into the bath, and supported so that the umbilical cord was free from tension. The stomach was exposed by removing most of the abdominal wall on the left side, making visible the rumen, reticulum and abomasum, but not the omasum. The left cervical vagus was exposed, but was not immediately cut.

Observations could then be made upon:

1. the presence or absence of spontaneous gastric movement before stimulation;
2. the effects of direct electrical stimulation of the stomach wall; and
3. the effects of electrical stimulation of the uncut vagus, or of the central and peripheral ends separately, after the vagus was cut.

The observations were not always made in the same sequence. Faradic stimulation was supplied by a Palmer’s induction coil with a pair of platinum wire electrodes. A constant coil distance of 10.5 cm. was used, at which a mild shock could be felt on the tongue. When observations on gastric movement were concluded the umbilical cord was tied off and the foetus removed and radiographed. In the foetuses used to study spontaneous swallowing the stomachs were opened to see where ink-stained fluid could be found. Before fixation each foetus was weighed and the crown-rump length measured as a check on the stage of gestation.

RESULTS

1. *Spontaneous swallowing* occurred in each of five foetuses of between 90 and 120 days. No information was gained as to the oesophageal reflex, the ink always being diffused throughout the stomach.

2. *Stimulated swallowing*. The 50-60-day foetuses responded to stimulation by slight movements of the tongue and jaws, but no throat movements were seen. Foetus 702A, of 70 days’ gestation, was seen to open its mouth before the amnion was ruptured. When the pipette was placed in the mouth the tongue curled up at the sides, and throat movements were clearly seen. No contrast material reached the stomach. 704A and B, also 70 days, showed much the same degree of activity, but without the tongue movement. 694A was approximately 74 days. The mouth was closed round the pipette and the tongue curled slightly, and although swallowing movements were not pronounced, a small quantity of barium was found in the reticulum. 693B, 80 days; 689A, 81 days; 692B, 90 days; and 695B, 100 days, all suckled, closing their mouths on the pipette and swallowing, and with one exception barium was later found in the reticulum and rumen. 689A received ‘Diodone’, iodine contrast-fluid, instead of barium, and the radiograph, taken 30 min. later, showed the liver to contain a quantity of radio-opaque material, while the stomach was not shown up. Analysis of the liver gave an iodine content of 0.009 g.% (wet weight), while the control (the liver of the twin foetus) contained only a trace (< 1 γ). This indicates rapid absorption from the stomach, but it is not possible to say with certainty from which part as, although at this age foetuses swallowed barium into the rumen and reticulum, the more diffusible suspension of Indian ink was found in the 90-day foetus to reach all four divisions. Of the six foetuses between 100 and 131 days none suckled as vigorously or swallowed as readily as those in the previous
3 weeks of development. Only two swallowed any barium; in 700, 110 days, the
barium was found in the rumen, reticulum, and body of the abomasum, which last
position might indicate the appearance of an oesophageal reflex. Barium was also
found mainly in the abomasum of the 131-day foetus. Finally, the 140-day foetus
was active and suckled well (this ewe received only 15 g. of urethane instead of the
normal dose of about 25); most of the barium passed to the abomasum, a small
quantity going to the reticulum, and only a trace into the rumen.

706 A, 100 days, was used to investigate spontaneous swallowing of amniotic fluid,
so it was not given barium, but after removing its head from the fluid to prevent
further swallowing, it was offered a finger to suck. This foetus showed a well-
developed pattern of suckling behaviour, closing its mouth firmly on the finger and
wagging its tail.

(3) *Spontaneous stomach movement*. Spontaneous movement was not often seen
on opening the abdomen, but it must not be assumed that such movement was
normally absent, as in the adult it is inhibited by the procedure of laparotomy. It
was never seen before 70 days, but at this age slight movements of the rumen were
seen in one foetus, and intestinal motility in another.

(4) *Direct stimulation*. Direct electrical stimulation had no visible effect until
54 days, at which time the ventral sac of the rumen contracted but the response did
not spread to any other part. In 55-day twins, stimulation of the ventral rumen sac
produced a contraction spreading to the dorsal sac. The dorsal sac also responded to
direct stimulation, but this response did not spread.

Table 2. *The first appearance of motor responses in the foetal stomach of the sheep*

<table>
<thead>
<tr>
<th>Foetal age (days)</th>
<th>Response to Direct stimulation</th>
<th>Peripheral vagal stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>40–50</td>
<td>Ventral rumen sac</td>
<td>o</td>
</tr>
<tr>
<td>54 approx.</td>
<td>Dorsal and ventral rumen sacs</td>
<td>o</td>
</tr>
<tr>
<td>55</td>
<td>Rumen sacs and localized reticulum contraction</td>
<td>o</td>
</tr>
<tr>
<td>60</td>
<td>All parts</td>
<td>Ventral rumen sac</td>
</tr>
<tr>
<td>70 onwards</td>
<td></td>
<td>All parts</td>
</tr>
</tbody>
</table>

At 60 days both rumen sacs responded separately. On one occasion the reticulum
also showed a sluggish and incomplete local response similar to a rumen contraction;
again a circular constriction of the intestine followed local stimulation. At 70 days
stimulation of the rumen sacs still had only a localized effect, but stimulation of the
reticulum caused a sharp reticulum contraction, followed slowly by a tetanic con-
traction of the ventral rumen sac, and sometimes by slow contraction of the body of
the abomasum. Stimulation of the abomasum itself caused only a limited local
response. From this age onwards local responses were usually strong in all parts of
the stomach, but their spread from one part to another was always uncertain, and
after 100 days it almost ceased. In one 100-day foetus the omasum was directly
stimulated, but without any visible effect.

(5) *Vagal stimulation*. The earliest stage at which the cervical vagus could be
dissected out was 55 days, and it was then so delicate that no attempt was made to cut it. Stimulation was carried out by applying the electrodes to the intact nerve, and no response was detected. At 60 days stimulation of the intact nerve caused only a slight contraction of the ventral rumen sac.

At 70 days and onwards the vagus was cut in the neck and a sufficient length was freed along each stump to allow it to be laid over the electrodes. Stimulation of the peripheral stump at all stages from 70 days onwards always elicited immediate total contraction of the reticulum. It usually brought about more slowly, and with a latent period, tetanic contraction of the rumen, especially strong in the ventral sac. This response was less constant than that of the reticulum.

The ventral rumen sac contractions, from their first appearance, were spiral. They caused the left side of the sac to become elevated into a peak, so that the shape of the sac came to resemble that of a top-shell (*Gibbula*). Once this contraction had appeared it often persisted for some minutes. After several stimulations it tended to become stronger and more persistent, and sometimes remained visible after the death of the foetus. The effect did not appear after 100 days.

In early stages the effect of peripheral vagal stimulation on the abomasum was usually to produce tetanic contraction of the body of the organ. At other times stimulation was followed by strong peristalsis of the whole abomasum, but this did not always occur, and on one occasion relaxation was seen. This irregularity is illustrated in the following extract from a protocol:

_Ewe no. 193, 2nd foetus, 80 days_

Peripheral stump of left vagus, first stimulation, 5 sec.
5 sec. Reticulum contracting strongly.
12 sec. Reticulum now relaxed. Strong contraction of whole body of abomasum.
15 sec. Ventral rumen sac beginning.
30 sec. Ventral rumen sac stronger. Pyloric antrum showing strong waves.
Two min. elapsed. Tonic contraction of abomasal body still present. Second stimulation, 5 sec.
5 sec. Reticulum contracted.
7 sec. Reticulum relaxing. Upper end of abomasum rather suddenly relaxed, but contraction persisted in a band at the 'antral furrow'.
25 sec. Ventral rumen sac contracting.
35 sec. Abomasal peristalsis recommenced.

Stimulation of the central stump of the cut cervical vagus was not productive of very definite results on the stomach, except that it was never followed by total contraction of the reticulum.

Contractions of the individual sacs of the rumen, of the abomasum, and surface movements on the reticulum were sometimes produced, and a spasmodic movement of the diaphragm often occurred.

In two foetuses only, at 120 and 131 days, the right cervical vagus was also cut after the left, and the effects were then studied of direct stimulation of the reticulum. In one, strong partial contractions involved about half the reticulum, but never the
Motor responses in the stomach of the foetal sheep 37

whole organ, although stimulation of the peripheral vagal stump still produced total contraction. In the other, similar partial contractions were brought about by stimulation of the anterior and dorsal part of the organ, but stimulation of the lower pole or of the posterior border against the anterior rumen sac gave practically a complete contraction. In fact, the results of direct stimulation seemed to be much the same in the presence or absence of the intact vagus.

(6) Other observations. The abomasum and top of the duodenum were seen on four occasions to be extremely active and the activity was analysed. The strong movement was never seen until some stimulation had been given, but a single vagal stimulation, either central or peripheral, as if ending an inhibition, often induced rhythmic movements which continued until the end of the experiment, except when modified by further stimulation. The movement seen in these foetuses was particularly interesting, as it has not hitherto been recorded in the adult sheep. Peristaltic waves passed down the abomasum in the typical manner; comparatively few waves passed over the body of the organ, although those which did so originated near the omaso-abomasal junction and passed right down to the pylorus. In between these, other waves originated at the incisura angularis and passed along the pars pylorica. Every third or fourth wave reaching the pylorus, whether it came from the top of the abomasum or not, was followed by a sudden contraction or short series of contractions of the pylorus and the visible part of the duodenum (about the first 1 or 2 cm.). Several of these contractions might occur in rather rapid succession, with intervals of four or five seconds. Each contraction seemed to occlude the lumen completely, and the wall was blanched, so that the appearance resembled that of a cardiac ventricular beat. It is therefore referred to as the duodenal beat. An example of the time intervals between successive beats, in seconds, is: 35, 15, 5, 15, 20, 5, 5, 40. The beating seemed to be related to the state of filling of the pyloric antrum. In two foetuses where the antrum was not distended single beats were seen to follow vagal stimulation. Altogether, single or multiple beats were seen to occur from 90 days to full term.

HISTOLOGY

Attempts were made in the first few experiments to perfuse foetuses with methylene blue by way of the umbilical vein, but this was abandoned. Satisfactory general views of the myenteric plexus were obtained from 60 days onwards by Gairns's gold chloride impregnation; this was less satisfactory in the earlier stages, possibly owing to a lack of differential metabolism in the nervous tissue. In the early stages the main fibre tracts and ganglia showed up quite satisfactorily in sections of material fixed in alcohol-formalin-acetic and stained with Heidenhain's haematoxylin.

A pair of 26-day embryos were obtained from a ewe which was a casualty; one was serially sectioned. No differentiation of muscle fibres was seen in the stomach, and no sign of nerves.

The next foetal stomach to be sectioned was one of 41 days' gestation. At this stage the inner, circular muscle layer was found to be differentiated, the main nerve trunks were present, and ganglia and tracts were seen outside the muscle layer.
By 50 days a few fibres of the outer, longitudinal muscle layer appear outside the nervous tissue, which is now much more prominent and is in fact most abundant in relation to the size of the organ. At 55 days there is quite a well-marked longitudinal muscle layer, and the proportion of muscle to nerve tissue grows rapidly greater from this point to the end of gestation.

**DISCUSSION**

Hamilton, Boyd & Mossman (1945) describe two stages in the development of motor mechanisms involving striated muscle, namely, a non-functional and a functional period. The latter is subdivided into a myogenic stage (when the muscle fibres can contract before the nerve supplying them can conduct), a neuromotor stage (in which direct stimulation of the centre of the nerve trunk causes muscular contraction), and finally a reflex stage. These authors point out that the 'three stages overlap to considerable extent, and some investigators (e.g. Straus & Weddell, 1940) think that the separation of a myogenic stage is artificial. Further, the reflex effects are not so localised as they are for a similar stimulus, in post-natal life.'

It is more difficult to recognize a reflex stage in smooth muscle, where autotthonous movement may occur in the absence of central control than in skeletal muscle, where an extrinsic stimulus is needed for contraction. Our observations have not provided definite evidence of the initiation of a reflex stage, except the appearance of the oesophageal reflex.

The earlier stages of functional development in the sheep's stomach appeared to be identical with those described by Hamilton et al. (1945). The non-functional period ended with the differentiation of the longitudinal muscle layer, between the 50th and 54th days of gestation. The myogenic stage had commenced by the 54th day, when the rumen was able to contract in response to direct stimulation. At 60 days the myogenic might be said to overlap the neuromotor stage, as movement of the ventral rumen sac could be elicited by vagal stimulation, while the dorsal sac and the reticulum were still in the stage of myogenic activity, brought about only by direct stimulation of the muscle. Although response both to direct and to vagal stimulation appeared first in the ventral rumen sac, no precocity in development of this part was detected histologically.

Whereas up to 60 days the only movements seen were direct responses to stimulation, from 70 days onwards sustained movements, such as successive contraction waves, were seen to occur. Vagal stimulation after this time affected the whole stomach, and the types of response to it resembled those of the adult stomach. Moreover, Barcroft (1946) first obtained evidence of vagal effects on the heart at 77 days from the right vagus, and at 81 days from the left. It seems likely that this stage, when adult responses are obtained, is akin to the development of the reflex stage in skeletal muscle. Further work would be necessary to discover whether the spread of contraction from one part of the stomach to another, which occurred at 70 days, involved nervous elements. Probably it was partly due to a simple spread of the electrical impulse across the small organ, and the decrease in spread after 100 days was a result of the increasing size of the stomach.
There was, however, one more constant finding which continued to full term, namely, that total contraction of the reticulum resulting from stimulation was nearly always followed, after a latent period, by peristaltic movement of the body of the abomasum.

Further work is required on the sensory aspect of the reflexes controlling gastric movement, especially in view of the varying results of stimulation of the central end of the vagus. The motor activity of the vagus is indicated by the results described above, showing that major movements are possible from approximately the time when the vagus becomes functional, and it has been confirmed by bilateral vagotomy in lambs, after which operation all major rumen and reticulum movements cease.

Suckling is a complex pattern and is built up over a long period of development. Responses of the mouth and tongue to touch stimuli were not seen until the 50th day, but Barcroft & Barron (1939) saw them as early as the 41st day, and they also saw 'throat movements... which indicated the foetus is sucking' at 49 days. We failed to detect them until the 70th day, by which time there was an external appearance as of sucking and swallowing, although the first foetus which actually swallowed was 4 days older. The ability to suck quite strongly was in evidence by 80 days, but it was 100 days before tail-wagging entered into the complex, and 110 days when there was any sign of oesophageal groove closure. According to Barcroft & Barron (1939) 'the effect of the urethane on the behaviour of the foetus becomes more marked as the age of the foetus increases'. As we were using urethane as an adjunct to the spinal anaesthesia, a full dose was not needed, but the foetuses were increasingly affected in the later stages. They were never anaesthetized, but were sluggish, and this accounts for the difficulty of obtaining suckling responses. Since the ewe only received 15 g. of urethane instead of 25–30 g., the oldest foetus of all, 140 days, was less affected, and it sucked readily, exhibiting a functional oesophageal reflex. Watson (1944) has shown the oesophageal reflex to be closely linked with the pattern of behaviour indicative of 'pleasure in sucking'. We have never actually watched the effect on the groove of vagal stimulation, but bilateral vagal section in suckling lambs abolishes the reflex, and this suggests that the vagus provides motor control for this as for other gastric movements. It is hoped to make a further study of the level of central nervous control and, if possible, the sensory arc.

**SUMMARY**

The state of motor activity of the stomach was studied in a series of foetal sheep. The non-functional period was found to end between the 50th and 60th days of gestation. Sustained activity was preceded by short periods of myogenic and neuromotor activity, and was not in evidence until the 70th day. Foetal swallowing commenced soon after this, but the pattern of suckling behaviour developed gradually over an extended period. The motor mechanisms mediated by the vagus were studied by section and electrical stimulation of the nerve.
The authors are indebted to Miss B. W. Simpson for iodine estimations, and to Mr D. Benzie for the radiography. They wish to express their thanks to the technical staff of the Physiology Department, and especially to Mr W. Brown.

REFERENCES