

signed to preclude chance intervention of *Bact. tumefaciens* or any similar organism.⁶

Bacteriological studies are being made of a series of these root-stimulating organisms in comparison with various soil organisms and *Bact. tumefaciens* isolated from typical crown-galls on apple. Although this work is incomplete, the data available have already established certain important differences between the root-stimulating cultures and the typical, gall-inducing *Bact. tumefaciens* of Smith and Townsend. These differences relate both to physiology and pathogenicity and are probably of specific rank.

The results thus far obtained give promise of elucidating at least one phase of the hairyroot question. At the same time they seem to open problems of much potential scientific and practical interest. What is the nature of the relationship of these root-stimulating organisms to the plants with which they are associated? Is it harmful or beneficial to the plant? If harmful, may these organisms be controlled economically; if beneficial, may they be adapted to use in plant culture to facilitate root development?

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THE MECHANISM OF PELLET FORMATION IN THE GREAT HORNED OWL (*BUBO VIRGINIANUS*)¹

It is well known that many birds, at varying intervals after feeding, regurgitate pellets composed of bones, fur, feathers and other indigestible materials, compactly arranged and free from digestible constituents as shown by the absence of any evidence of decomposition even after several days. The detailed report by B. P. Reed² on the formation of these pellets in the great horned owl suggested the use of this species as experimental subjects in an attempt to gain some information on the mechanism involved in delaying the passage of certain food materials through the stomach as occurring in higher forms.

⁶ On May 3, 1928, when the present paper was being prepared for publication, the writers were pleased to learn through correspondence that Miss Nellie A. Brown, of the Bureau of Plant Industry of the United States Department of Agriculture, in unpublished work, has induced the development of hairyroot on Paris-daisy plants which were inoculated with bacteria isolated from hairyroot on apple.

¹ From the Hull Physiological Laboratories, University of Chicago, and the Department of Physiology, Baylor Medical School.

² B. P. Reed, 1925, *The Auk*, xlii, 14.

Of all the common birds displaying this phenomenon the owl forms the largest pellets and so seemed best adapted to the purpose.

Seven nestlings were secured at about the age of four weeks and raised in captivity so that they might become accustomed to handling. All but one became fairly tractable when properly handled. None was submitted to any experimental procedure until about six months old, at which time they had attained adult development.

One method of study was by fluoroscopic examination after ingestion of barium paste or of food mixed with paste. In the latter procedure freshly killed rats or guinea-pigs were cut into pieces and these rolled in thick paste, or raw liver was similarly treated; in other experiments finely minced meat, containing bones, hair or feathers, was mixed with barium paste and enclosed in small gauze bags and a definite number of these swallowed by the bird. It was then possible to count and locate these under the fluoroscope. These bags were invariably regurgitated as pellets entirely freed from digestible material in from twelve to twenty hours, the normal range of time involved in this process.

Another procedure involved aspiration of gastric juice at various stages of digestion. Usually not more than one or two cubic centimeters of fluid could be obtained, indicating that gastric secretion is not profuse at any time in this species. This material was tested for free and total acidity and peptic activity. All samples were highly viscid, dark in color, malodorous but never putrid.

Freshly regurgitated pellets were extracted with a measured quantity of water, squeezed dry and the extract filtered and tested as above, acidity being calculated for the original moisture content. These contained from two to three cubic centimeters of juice.

Two birds were killed at different stages of digestion of a meal consisting of the chopped body of a freshly killed rat, and the stomach contents examined both grossly and chemically.

Two trials at the preparation of gastric fistulae failed, but a third was successful, the bird continuing in a healthy state for several months and was finally killed for examination. But at no time was it possible to obtain enough gastric juice from the fistula for examination during digestion. Only a few drops were ever observed to escape from the opening at any time. This is a further indication that gastric secretion is not profuse in this species.

Post-mortem examination showed the pyloric opening of the stomach to be only about 1 mm in diameter, and in the normal position under the fluoroscope it was always found to lie on a level with the opening of the esophagus, so that practically the entire content

of the stomach, even when greatly distended, lay below the level of the pylorus. Under the fluoroscope movements of the pylorus were never observed. Apparently a thin stream of liquid flowed through without obstruction. The pyloric opening appeared to be about the same diameter as found post-mortem. These facts indicate that there is a mechanical bar to passage of anything but finely divided material from the stomach to the intestine.

Incidentally, it was found that when the thorax was opened under an anesthetic the bird continued to breathe with no difficulty by reason of the fact that the lungs were so attached to the ribs that costal movements distended them without any involvement of pressure changes. Birds in this condition were observed to continue adequate respiration for as long as thirty minutes.

GASTRIC MOTILITY

Two of the subjects were especially tractable and when blindfolded could be observed repeatedly for brief periods over several hours or an entire day on the fluoroscope. It is believed that the gastric motility observed under these conditions was normal in every way and not inhibited, since pellet regurgitation was not delayed in any case.

At no stage of digestion was any extensive motility observed. By sketching on the screen the outlines of the stomach at successive observations it was possible to compare the position and shape at different stages. As emptying progressed there was always reduction in diameter in every direction. Such movements as were observed consisted of very gentle peristaltic waves beginning near the cardia and progressing continuously to the region of the pylorus. This is in keeping with the anatomical structure of the stomach. The musculature is very thin and hence not adapted to any great degree of motility.

Apparently, a mechanism similar to vomiting in higher forms is involved in regurgitation, since the subjects show evidence of nausea for fifteen or twenty minutes before the mass is discharged. The feeble musculature of the stomach precludes the involvement of any great force. However, the esophagus is very large and distensible, so that there is no apparent bar to easy discharge. On a few occasions when it was observed that regurgitation was imminent, very gentle pressure with the hand on the abdominal wall was sufficient to cause immediate expulsion of the pellet. In spite of feeble gastric motility during digestion, it is apparent that the musculature is sufficiently active to discharge indigestible masses. No information is available at present as to the nature of the reflex mechanism involved in the process.

ACIDITY

No free acidity was present in gastric contents at any stage of digestion nor in the extracts of pellets. Twenty-eight samples of gastric contents and six pellets were tested, using methyl orange as an indicator. Total acidity in one sample taken five hours after eating was found to be 0.155 per cent.; in another sample taken two and one half hours after a meal total acidity was 0.437 per cent.; four hours after, 0.282 per cent.; in case of the entire stomach contents secured by killing the bird four hours after feeding, total acidity was 0.063 per cent.; a pellet regurgitated twenty hours after feeding was extracted and total acidity was found to be 0.2 per cent. while in another regurgitated by the same bird after a comparable period total acidity of the extract was found to be only 0.07 per cent. These figures are typical of the many determinations. The absence of free acidity possibly accounts for the fact that bones in the pellet masses showed no evidence of corrosion. The ends were sometimes crushed and broken, but this apparently occurred before swallowing as the birds are accustomed to crush food masses in the beak. The broken edges were always sharp and clean and when examined under a low-power microscope showed no evidence of corrosive action. Furthermore, pigment in hair and feathers appeared to be undisturbed.

Peptic activity was found to be generally about three times as potent as that of dog gastric juice at comparable stages of digestion.

Observations made by killing during digestion and examining the gastric contents showed that there was no mixing of materials after swallowing, as small masses of different colored hair retained their original relative positions. The outside of the pellets consisted of hair and bones cleanly freed from digestible materials, while there was progressively less digestion in evidence toward the center of the mass.

These observations indicate that the mechanism of pellet formation involves three general factors: first, the mechanical factor of high placement and small size of the pyloric opening; second, feeble gastric motility which would preclude stirring up or freeing of hair or feathers enmeshed in the whole mass; third, potent peptic activity which would readily digest all other material free from hair, feathers and bones, and liquefy it so that pyloric passage would be facilitated.

So far as present evidence indicates there is no relation between pellet formation and the selective passage of food materials from the stomach as occurring in higher forms.

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