THE VERTEBRATE SMALL INTESTINE AS AN ENVIRONMENT FOR PARASITIC HELMINTHS

By

CLARK P. READ, JR., PH.D.
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CLARK P. READ, JR.
INTRODUCTION

MANY persons, even biologists, who are not directly concerned with study of the physiology of the digestive tract, tend to think of the alimentary canal in simplified terms. It is often regarded only as a tube through which the food passes, into which enzymes and a few other substances are secreted to aid in the breakdown of food molecules too complex to be absorbed, and from which the digested food materials are absorbed. Actually, the events occurring in the intestinal tract are much more involved than this. Enough observations have been recorded by workers in several fields to indicate that the biology of the intestinal lumen is marvelously complex and that the lumen is in a very dynamic relation with the body tissues. Recognition of this fact, and a more thorough knowledge of the peculiarities of the intestine as an environment are necessary in order that parasitologists may have an adequate understanding of the physiology and ecology of parasites and of host-parasite relations. This is particularly true of the parasites (Cestoda and Acanthocephala) which inhabit the lumen of the intestine and completely satisfy their food requirements by absorption through their external surfaces.

This paper is presented as a general survey of the knowledge available on what seem to the writer to be those aspects of gut physiology of most importance to the parasitologist. The breadth of the subject matter is such that a comprehensive review is impossible in the space available. But references have been made to most of the important contributions that the writer has been able to ferret out of the literature so that the interested parasitologist may more easily delve deeper into the subjects briefly discussed. Liberal use has
been made herein of a number of excellent review articles
dealing with various phases of the subject. These are cited
at appropriate points. Undoubtedly, however, many aspects
of certain topics are inadequately documented and are thus
open to criticism. The writer is primarily a helminthologist
and must beg forgiveness of the physiologists and bio­
chemists for sins of omission. Nevertheless, it is felt that
enough information is presented to indicate the state of
present knowledge on the subject; and it is hoped that stu­
dents of helminthology may be stimulated to further ap­
preciation and study of the vertebrate gut as an environment
for parasites.

It is difficult to make many generalizations concerning the
intestinal physiology of vertebrates. In many cases fragments
of knowledge are highly suggestive, but few of the subjects
herein dealt with have been comprehensively investigated.
The indulgence of the reader is sought if in some places the
writer follows Shakespeare’s advice to “piece out our im­
perfections with your thoughts.”
I. THE GENERAL CHARACTERISTICS OF THE INTESTINAL ENVIRONMENT

The pH of the gastrointestinal tract is probably its most extensively investigated physico-chemical characteristic (excluding digestive enzymes). The subject has been comprehensively reviewed elsewhere (64, 65, 228, 321). Considerable evidence has accumulated to show that there is a certain constancy of pH in various sections of the small intestine (111, 330, 409, 411, 412, 413, 515). It seems to be agreed that the hydrogen ion concentration decreases progressively as the ileocecal valve is approached. Obviously, the proximal portion of the duodenum which receives the acid chyme from the stomach may undergo some rather pronounced fluctuation in pH. However, the bile and the pancreatic and intestinal juices exert a powerful buffering effect, so that the contents of the intestine are quickly brought to a pH near neutrality. There is evidence that the pH near the mucosa is relatively stable. This topic will be discussed more fully in Section V.

Few workers have studied the osmotic pressure of the contents of the intestine. Hobson (228) summarized most of the available data, reproduced here in Table 1. It has been generally accepted that the contents of the gut are usually hypertonic as compared with the blood of the vertebrate concerned (65, 228, 464). As an exception, Davey (107) reported a close similarity in the freezing points of the blood and the contents of the rumen, reticulum, and abomasum of lambs.

A recent study by Follansbee (158) is of great interest. Follansbee showed that the osmotic pressure of the intestinal
content of the rat is very close to that of blood from the same animal. Furthermore, it was demonstrated that after collection the osmotic pressure of the intestinal contents rather rapidly becomes hypertonic. Evidence was presented to show that these changes are due to the activity of microorganisms; but this source of error was largely eliminated by promptly poisoning the material with mercuric chloride at the time of collection. These observations may throw doubt on at least some of the earlier observations, and indicate that study of the osmotic pressures of the intestinal contents in vivo would be highly desirable.

There can be little doubt that there may be marked fluctuation in the osmotic pressure in the stomach, and perhaps in the duodenum, after the ingestion of water, but there is evidence that fluctuations may be slight and of short duration in the remainder of the gut. Water is absorbed rather rapidly from the intestine (158).

The gases present in the intestine have been studied by a number of investigators. Some of the data are summarized in Tables 2 and 3. The amount of oxygen in the gut has claimed a great deal of attention from parasitologists be-

<table>
<thead>
<tr>
<th>Animal</th>
<th>$\Delta(°C)$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse</td>
<td>0.69</td>
<td>(445)</td>
</tr>
<tr>
<td>Horse</td>
<td>0.755</td>
<td>(448)</td>
</tr>
<tr>
<td></td>
<td>(0.674–0.850)</td>
<td></td>
</tr>
<tr>
<td>Horse</td>
<td>0.69</td>
<td>(130)</td>
</tr>
<tr>
<td>Pig</td>
<td>1.02</td>
<td>(445)</td>
</tr>
<tr>
<td></td>
<td>(0.73–1.30)</td>
<td></td>
</tr>
<tr>
<td>Pig</td>
<td>0.869</td>
<td>(228)</td>
</tr>
<tr>
<td></td>
<td>(.0783–0.940)</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>0.815</td>
<td>(445)</td>
</tr>
<tr>
<td><em>Scyliorhinus caniculus</em></td>
<td>2.30–2.65</td>
<td>(444)</td>
</tr>
</tbody>
</table>
cause of the significance that its presence or absence might have in formulating hypotheses concerning the aerobic or anaerobic metabolism of parasites. There is good evidence that oxygen is present in small amount in the contents of the small intestine. This evidence has been discussed in detail elsewhere (63, 64, 65, 228).

There is some variability in the amounts of oxygen reported by various workers. This may be due to difference in techniques, state of digestive activity, or, as Brand and Weise (67) pointed out in the case of the pig, swallowing of air during slaughtering. There are also, no doubt, species

<table>
<thead>
<tr>
<th>Animal</th>
<th>CO₂</th>
<th>O₂</th>
<th>CH₄</th>
<th>H₂</th>
<th>N₂</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>13.56</td>
<td>0.19</td>
<td>2.83</td>
<td>7.72</td>
<td>75.71</td>
<td>(485)</td>
</tr>
<tr>
<td>Dog</td>
<td>15.92</td>
<td>0.29</td>
<td>—</td>
<td>26.48</td>
<td>57.28</td>
<td>(485)</td>
</tr>
<tr>
<td>Goose</td>
<td>2.04-87.83</td>
<td>0-3.62</td>
<td>0-13.51</td>
<td>0.72-20.06</td>
<td>67.92-85.28</td>
<td>(485)</td>
</tr>
<tr>
<td>Pig</td>
<td>2.16-79.89</td>
<td>0.08-3.62</td>
<td>0.2-28.29</td>
<td>4.25-39.56</td>
<td>2.02-78.86</td>
<td>(485)</td>
</tr>
<tr>
<td>Horse</td>
<td>15-43</td>
<td>0.57-0.76</td>
<td>0</td>
<td>20-24</td>
<td>37-60</td>
<td>(486)</td>
</tr>
<tr>
<td>Rabbit</td>
<td>75</td>
<td>0</td>
<td>2</td>
<td>18</td>
<td>6</td>
<td>(486)</td>
</tr>
<tr>
<td>Cattle, sheep, and goats</td>
<td>62-92</td>
<td>0</td>
<td>0.04-6.6</td>
<td>0-37</td>
<td>1</td>
<td>(486)</td>
</tr>
<tr>
<td>Pig</td>
<td>5-28</td>
<td>5.0</td>
<td>0</td>
<td>0</td>
<td>74-92</td>
<td>(304)</td>
</tr>
<tr>
<td>Pig</td>
<td>—</td>
<td>0.4-8.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>(67)</td>
</tr>
</tbody>
</table>
differences which may involve such factors as diet. From Table 3 it would seem that ruminants tend to have much less oxygen in the intestine than do other animals. The pig may have somewhat more than other species. Slater (462) assumed that the intestinal walls give off oxygen to the intestinal contents during digestion. There is no evidence that this is true. Long and Fenger (304) found that the oxygen was lowest when digestive activity was high. The only reason for supposing that more oxygen might enter the intestine during digestion would rest on the assumption that the increased flow of fluid secretions into the gut might carry in additional oxygen. However, there is no real basis for assuming this to be true; bile, for example, contains little oxygen (67, 72, 73). Furthermore, the fluid intestinal masses contain only small amounts of oxygen, as shown in Table 3 reproduced from Brand and Jahn (65).

The data of McIver, Redfield, and Benedict (328) indicated that oxygen diffuses into the intestine from the mucosa. Rogers (418) recently measured the oxygen tension in the contents, close to the mucosa, of the small intestine of sheep and rats. He reported oxygen tensions of 4.0 to 12.7 mm. Hg in the sheep and 7.9 to 30.2 mm. Hg in the rat. It is obvious that worms living near the mucosa have access to larger amounts of oxygen than might be expected from analyses of the intestinal contents. This point will be discussed further in a later section of this paper.

It was pointed out by Rogers (418) that utilization of oxygen by intestinal parasites might tend to lower the oxygen tension in the gut. However, no significant difference was observed in the tensions in parasitized and unparasitized animals. This may not be surprising if we consider the probable kinetics involved. It has been shown that most gas exchanges
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between the lumen of the intestine and the blood are due to
the physical process of diffusion (328). One of the criteria
for defining physical diffusion is that the exchange of a gas
which leads to establishment of an equilibrium is deter-
mined by the law that the rate at which a gas passes through
a membrane will be proportional to the difference in the
pressure of the gas on the two sides of the membrane. It fol-
 lows from this that removal of oxygen by a parasite would
simply result in an increase in the rate of passage of oxygen
into the intestine. Of course, actual diffusion equilibrium
between gut and mucosa is probably never attained because
of other factors, such as removal of oxygen by bacterial ac-
tion. In this connection, there is also the possibility that
intestinal helminths may induce a hyperemia of the intestinal
mucosa which might result in increased oxygen tensions in
the intestinal lumen.

As pointed out by Brand and Jahn (65) nothing is known
of the effect on intestinal helminths of gases other than
oxygen. Brand and Jahn postulated that since carbon dioxide
is usually present in the environment in high concentration,
intestinal helminths may depend on this gas as an intracellu-
lar buffer. The writer has recently obtained evidence that
carbon dioxide is utilized in a different manner in the me-
tabolism of some cestodes. The tapeworm *Hymenolepis
diminuta* may form at least a portion of the end products of
its metabolism by chemical reactions involving a carbon
dioxide fixation (393). Thus, carbon dioxide may have more
than one role in the physiology of intestinal worms. Brand
and Jahn (65) assumed that most of the carbon dioxide of
the intestinal lumen arises from bacterial metabolism. This
may not necessarily be so. Fairly large quantities of carbon
dioxide may arise from the action of acid on the secretions
of the gut, which contain appreciable amounts of bicarbonate. Moreover, carbon dioxide is present as such in the intestinal secretions. Support for this statement may be found in the discussions below of specific secretions. In addition, carbon dioxide may diffuse into the intestine from the mucosa since it diffuses more readily than the other intestinal gases (328).

The oxidation-reduction potential of the contents of the small intestine has apparently been little investigated. The writer has found data reported from only one animal, the rat. Jahn (239) reported Eh values of $-195$ to $-200$mv. for the large intestine and cecum. Bergeim et al. (47) reported values of $+150$mv. for the stomach, $-100$mv. for the upper and lower small intestine, and $-200$mv. for the cecum. These variations correspond with the increasing number of bacteria in different intestinal segments. Widely varying diets gave similar results. Inclusion of sulfonamides in the diet had little effect, but high lactose or raw apple diets had a marked effect, producing positive Eh values in the small gut (47).
II. THE CONTRIBUTIONS TO THE SMALL INTESTINE

After surveying the general characteristics of the intestinal environment, we may study more particularly the various materials entering the intestine from the stomach and from the glandular structures associated with the small gut (liver, pancreas, and mucosa). It seems desirable to determine what knowledge we have of the secretions, with special reference to their role in the conditioning of the intestinal lumen as an environment for parasites.

A. Contributions from the Stomach

The chyme passes into the small intestine from the stomach with varying degrees of regularity, depending in part on the feeding habits of the species involved. It includes partially digested foodstuffs such as proteoses and peptones, fats, some fatty acids, glycerol, inorganic ions, undigested residues (e.g. cellulose), water, and certain materials present in the secretions of the gastric mucosa. There is undoubtedly great variability in the quantity and quality of most of these constituents. However, the components of the gastric secretion have a certain degree of qualitative constancy.

Although the subject of gastric secretion is beyond the scope of this review and has been extensively reviewed elsewhere because of its clinical importance (29), a few remarks on the composition of the juice which passes on into the lower gut are pertinent. The inorganic constituents of gastric juice have been rather thoroughly investigated, and Babkin (29)
The Rice Institute Pamphlet has reviewed the available data. HCl, Cl⁻, HCO₃⁻, K⁺, Na⁺, and Ca⁺ are the principal inorganic constituents.

The nitrogenous components of the juice are more complex than might be expected. Komarov (267) has described the partition of nitrogen in canine gastric juice. His data are reproduced in Table 4. Some studies have been made on the digestibility of gastric “mucin.” It was reported that hog “mucin” was partially digested \textit{in vitro} by trypsin or erepsin (16); \textit{in vivo} “mucin” was digested to a much greater extent

<table>
<thead>
<tr>
<th>Product</th>
<th>Sample A (4 liters)</th>
<th>Sample B (6 liters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>18.00</td>
<td>19.90</td>
</tr>
<tr>
<td>N. P. N.</td>
<td>9.80</td>
<td>10.90</td>
</tr>
<tr>
<td>Urea</td>
<td>0.16</td>
<td>0.11</td>
</tr>
<tr>
<td>Creatine and Creatinine</td>
<td>0.09</td>
<td>0.11</td>
</tr>
<tr>
<td>Bases:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5.37</td>
<td>6.59</td>
</tr>
<tr>
<td>Volatile</td>
<td>1.73</td>
<td>2.55</td>
</tr>
<tr>
<td>Non-volatile</td>
<td>3.59</td>
<td>4.04</td>
</tr>
<tr>
<td>Purines</td>
<td>0.11</td>
<td>0.10</td>
</tr>
<tr>
<td>Histidine-Arginine</td>
<td>1.56</td>
<td>1.77</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.88</td>
<td>2.17</td>
</tr>
<tr>
<td>Mono-amino acids</td>
<td>0.70</td>
<td>1.02</td>
</tr>
<tr>
<td>Humic acid</td>
<td>3.73</td>
<td>3.29</td>
</tr>
</tbody>
</table>

(17). Mahlo and Mulli (308) reported that gastric mucus from the human being was digested by juice from the duodenum. Some of the products of digestion were cysteine, tyrosine, and histidine.

Little is known of the vitamins present in the gastric juice. Vitamin B₁₂ is apparently a normal constituent of human juice (528). There is circumstantial evidence that thiamine is a component (324).

The question naturally arises as to what these things may
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mean to the parasitologist. The significance lies mainly in the fact that intestinal helminths may have access to a wide variety of substances which are not necessarily components of the host diet. In the discussion to follow this will become more apparent.

B. THE SUCCUS ENTERICUS

As the name implies, the succus entericus is the juice secreted by the glandular intestinal mucosa. It is not to be inferred that the succus is secreted by a single type of glandular tissue. As we shall presently see, it is undoubtedly a composite, a mixture of secretions. (The term "secretion" as used in this instance includes the movement of substances entering the gut by diffusion as well as those entering by active transfer mechanisms requiring the expenditure of energy.)

Most of the experimental study of intestinal secretion has been carried out on the dog. Two types of experiment have been widely used: The "acute" preparation in which a loop of the gut is studied for a short period, and the "chronic" fistula where a loop of the gut is isolated surgically and made to empty externally. The techniques of these preparations have changed little in the last twenty years. Details of the methods for preparing various fistulae may be found in the works of Markowitz (312) and Babkin (27), who also furnish many additional references on the subject.

For purposes of convenience in discussion, the small intestine is separated into two parts, the Brunner's gland region of the duodenum, and the remainder of the small intestine or small intestine proper. On the basis of distinct
differences in structure and function, such a separation seems justified.

The Brunner's Gland Area

Anatomically, this region appears to be continuous with the glandular pyloric stomach. Brunner's glands extend into the submucosa of the proximal portion of the duodenum and are composed of ramifying tubules which empty by many ducts into the bases or sides of the crypts of Lieberkuhn in the mucosa. The alveoli of the glands are generally of the mucous type; in the rabbit and hare serous cells are also present (197). The anatomy of this intestinal region will not be considered further in this paper; many textbooks furnish further details.

Duodenal juice was apparently first collected and described from the horse by Colin in 1854 (94). Little or no further investigational work was done on the juice until 1902 when Ponomarew used the fistula technique to study the gross physical properties of the duodenal juice of dogs (387). Florey and Harding (153, 153a, 153b) and Wright et al. (529), using both "acute" and fistula preparations, made extensive investigations of the upper duodenal secretions of the cat, dog, goat, sheep, rabbit, and pig. From clean fistulae the juices were clear and colorless or sometimes faintly yellow. On centrifuging, some juices yielded a deposit which consisted of mucin and cell debris. While the juices from all species were mucoid, the consistency (viscosity) varied from species to species. Juices from the rabbit, goat and sheep were more viscous than those collected from the pig, cat, and dog.

The chemical composition of secretion from the area of Brunner's glands is not well known. Ponomarew (387) reported that duodenal juice from the dog was alkaline and
estimated the alkalinity as equivalent to .09 to .15 per cent Na₂CO₃. Florey and Harding (153, 153a) found that juice from the goat was weakly alkaline, 1.0 ml. of juice being equivalent to .04 ml. of N/10 Na₂CO₃; on the other hand, these workers found that 1.0 ml. of rabbit juice (fistula) was equivalent to as much as 0.75 ml. of N/10 Na₂CO₃. The alkalinity of cat, dog, and pig juice was intermediate, 1.0 ml. being equivalent to 0.25 to 0.5 ml. of N/10 Na₂CO₃. These juices had an alkaline pH at the end of the collection period, probably due, as these workers point out (155), to loss of CO₂ during collection.

Wright et al. (529) observed that mucins in the duodenal juices of the rabbit, sheep, and goat were precipitated by acetic acid, while addition of the acid to juices collected from the pig, cat, and dog produced only a faint haze. It should be remarked that little is known of the chemical nature of animal mucins. The above mentioned observation may indicate species differences. This topic should be investigated further since it may have a bearing on host specificity of parasites (3, 5).

Havard’s work (212) has indicated that the buffering power of the juice is mainly dependent on bicarbonate and not on mucins. It has long been thought that mucous secretions protect the underlying mucosa. The alkalinity of duodenal juice suggests that the secretion of Brunner’s glands may protect the delicate villi of the proximal part of the small intestine from the damaging effect of the acid materials entering from the stomach. Villemin (504), after studying the anatomical relationships of Brunner’s glands, pointed out that they were not usually found past the entrance of the pancreatic duct, but were apparently independent of the opening of the bile duct. Villemin believed that the glands
were concerned in the neutralization of gastric juice. Florey et al. (154) produced evidence favoring this view by showing that the Brunner’s gland area in the pig is more resistant to gastric juice than are other parts of the small intestine.

The enzymes of the duodenum have been studied by making extracts of the mucosa and by examining secreted juice. We are mainly concerned with the latter. Ponomarew reported that juice from the dog contained invertase, lipase, amylase (irregularly), as well as pepsin-like and rennin-like enzymes (387); Pavlov and Parastschuk (377) confirmed the presence of the last two. Florey and Harding (153a) reported a pepsin-like enzyme in juice from goat and dog, and small amounts of lipase in the dog.

Wright et al. (529) have recently studied the enzyme content of juices from several species. These juices were spontaneously secreted, as well as produced by vagal stimulation, sympathetic section, and eserine administration. Good chemical methods and precautions against bacterial contamination were used in these experiments. Tests for proteases were consistently negative, no trace of pepsin-like, trypsin-like, or cathepsin-like enzymes being found. Di- and polypeptidases were found only when the juices contained significant amounts of cellular material. Traces of lipase were occasionally found. The importance of cellular content was demonstrated by the addition of cells to juice which had no dipeptidase or sucrase activity. Following the addition of cells from the intestinal villi the juice was capable of splitting dipeptides and sucrose. The juices from all species invariably contained amylase and were capable of activating protease activity in pancreatic juice (indicating the presence of enterokinase). Interestingly enough, the bacteriolytic lysozome was found in appreciable amounts in juice from
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the pig, but only in traces in juices from other species.

There is some evidence that nutritionally important factors may be secreted in the Brunner's gland area. Meulengracht and his co-workers (336-341) showed that preparations from different parts of the stomach and duodenal mucosa of the pig possess varying degrees of effectiveness in the treatment of pernicious anemia. They did not demonstrate the actual source of the active principle, but considered Brunner's glands as the most likely source in view of the anatomical similarity between these glands and those of the pyloric stomach. Goodfriend, Chain, and Florey (178) investigated the possibility of the actual secretion into the intestinal lumen of an anti-pernicious anemia factor. These workers used the reticulocyte response of guinea pigs as proposed by Jacobson (238) to test for anti-pernicious anemia factor in the duodenal juices of the pig, cat, rabbit, and goat. A reticulocytogenic agent was found in all cases. Its chemical nature is not known. The recent report of Wolf et al. (526) of the anti-anemia factor, Vitamin B-12, in fasting and histamine-induced gastric juice suggests that this substance may also be present in duodenal juice.

The amounts of juice secreted seem to vary somewhat with different species and is apparently not correlated with the size of the animal. Ponomarew reported that his fistula dogs produced .06 to 1.3 ml. an hour (387). Florey and Harding (153a) obtained 1.5 ml. an hour from a dog after feeding, while Wright et al. (529) obtained 1.5 to 2.0 ml. an hour from fully innervated dog fistulae. Fogelson and Bachrach reported as much as 8.8 ml. from a fed dog (157). Much larger quantities of juice are secreted by pigs, goats, and rabbits, which have a greater development of Brunner's glands. Wright et al. (529) obtained as much as 17 ml. an
hour from a fistula pig; one large goat secreted 150 ml. in 7 hours. These workers also reported relatively great production of juice in fistula rabbits.

The control of secretion in the Brunner's gland area evidently differs from the mechanisms controlling secretion in other parts of the small intestine. Florey et al. (155) point out that typical duodenal juice can be collected without mechanical stimulation, whereas other parts of the intestine only produce fluid after local stimulation (excluding "periodic" juice). Ponomarew (387) showed that introducing gastric juice, the products of gastric digestion of fibrin, or even normal saline into duodenal fistulae caused an increased flow of juice. Fatty materials had no effect. Florey and Harding (153, 153a,) found that HCl (N/10 or less) produced an increased flow of juice and a considerable degree of neutralization. Histological studies on the cat, rabbit, and guinea pig indicated that the passage of HCl through the duodenum exhausted the mucin-containing cells of the duodenal glands. The feeding activity of the animal is a stimulus for duodenal secretion, probably through production of the hormone duocrinin, produced in the intestinal mucosa (155, 157, 187). It is of interest to point out that few helminths live in the duodenum. Some trichostrongyles and Ascaridia are exceptions.

**The Small Intestine Proper**

The mucosa lining the small intestine is made up of tubular glands, the crypts of Lieberkuhn, and projecting villi. These structures are covered by a single layer of epithelial cells with occasional interspersion of other cells. These epithelial cells are simple cuboidal or cylindrical cells and goblet cells which are very numerous on the villi. Argentaffine cells scattered between epithelial cells are found in the
upper small intestine; cells with large eosinophilic granules, Paneth cells, are found at the bases of the crypts in some species. The many small cells lying between epithelial cells are apparently lymphocytes. According to Maximov and Bloom (317) these lymphocytes are known to migrate out into the lumen and return to the mucosa. This suggests that the region in the crypts may bear more similarity to the internal milieu of the animal than might be surmised. The histological details of the gut are thoroughly discussed by Patzelt (374) and Dawson (109).

Our knowledge of the microanatomy of the mucosa is thus rather extensive. In contrast, our knowledge of the function of the several cell types of the mucosa is rather scanty. For example, it is not known whether the villi absorb and the crypts secrete, or whether each is able to function in both capacities. The latter view seems to the writer the more probable since Osterhout (369) has shown that a single cell may absorb and secrete simultaneously. Virtually nothing is known about the function of the Paneth and argentaffine cells. Hellman (216) suggests that the lymphocytes function in defense against harmful substances in the gut. However, Sanders and Florey (433) showed that all intestinal lymphatic masses, Peyer's patches, can be removed from a rabbit or a rat with no evident detrimental effect and without subsequent development of compensating lymphatic tissue. This entire matter is, therefore, still an open question.

The physical characteristics of the intestinal juice have been repeatedly recorded. There is fairly general agreement that the juice consists of a yellowish, clear, or opalescent fluid portion and a flocculent part mainly composed of mucus, epithelial cells, cholesterol crystals, and microorganisms (155). The juice has an aromatic odor and is nearly neutral in reaction.
According to Bickel and Kanitz (50) the pH of juice from man is 8 to 9. Amberg and Sawyer (15) reported a similar pH range for dog juice. However, deBeer et al. (111), using better collection and chemical methods, found pH values of 6.30 to 7.28 for jejunal juice and 7.61 for the ileal juice from dogs, Shiffrin and Nasset (456) reported 6.9 to 8.6 to be the pH range of jejunal and ileal secretions of dogs.

The chemical composition of intestinal juice has been investigated by several workers. Data on the inorganic constituents of fistula juice are summarized in Table 5. Study of these data indicates that the osmotic pressure of intestinal juice is very similar to the osmotic pressure of blood serum. Rosemann (425) reported that intestinal juice gave a freezing point depression of $-0.60^\circ$ C, which is essentially the same as that of blood. Attention should be directed to the conclusion of Valette and Cavier (502, 503) that the composition of the secretion from a jejunal loop was essentially that of a transudate of blood plasma with the important exception that it was practically sugar-free, and to the report of McGee and Hastings (323) that samples of juice from the jejunum of fasting humans were isotonic with serum. When hypertonic salt solutions were injected intravenously a rise in the osmotic pressure of the ileal secretions was observed (507). It has been demonstrated that the intestinal mucosa may be grossly injured by excessively hypotonic solutions (115, 307, 399).

Some experimental data from this laboratory (393) serve to support the concept that at least some of the components of intestinal juice are directly derived from the blood plasma. Three albino rats with ileostomy fistulae were used in these experiments. Radioactive phosphorus ($P^{32}$) was administered to each animal intraperitoneally as a trace amount of sodium
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At intervals after administration of the labeled phosphorus, samples of juice were taken from the fistula and a blood sample was obtained simultaneously from the tail vein. Maximal specific activity (counts/min./mg. P) was found in the blood samples taken 13 to 16 minutes after injection of the isotope. The changes in specific activity of the ileal secretions of each animal closely paralleled the changes occurring in the blood of the animal. A typical example of the changes in the radio-phosphorus content of the intestinal secretion is shown in Figure 1. The time required for attainment of maximal activity in the blood in these experiments corresponds closely with the data of Kjerulf-Jensen (260) who observed that the maximum P³² content of rat plasma was reached 14 minutes after intra-peritoneal injection.

DeBeer et al. (111) and Herrin (219) demonstrated that the cationic concentration is relatively constant from one secretion to another. DeBeer and his colleagues showed that intravenous administration of sodium chloride to fistula dogs

### Table 5. Inorganic Components of Intestinal Juice

<table>
<thead>
<tr>
<th></th>
<th>Na</th>
<th>Mg</th>
<th>K</th>
<th>Ca</th>
<th>Cl</th>
<th>PO₄</th>
<th>HCO₃</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunum, Dog</td>
<td>126-156</td>
<td>2-1.9</td>
<td>4.8-10.2</td>
<td>1.6-5.4</td>
<td>141-153</td>
<td>1.2-7.9</td>
<td>5.2-30**</td>
<td>(111)</td>
</tr>
<tr>
<td>Jejunum, Dog</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(as P)</td>
</tr>
<tr>
<td>Jejunum, Dog</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(413)</td>
</tr>
<tr>
<td>Ileum, Dog</td>
<td>146-156</td>
<td></td>
<td>4.7*</td>
<td>5.5*</td>
<td>68.1-87.9</td>
<td>69.8-97.3</td>
<td>4.11-17.61</td>
<td>(111)</td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(111)</td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(413)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ca. 8</td>
</tr>
</tbody>
</table>

* Single determination.
** As total CO₃.
caused an increase in the Na\(^+\) and Cl\(^-\) concentrations in ileal and jejunal secretions. A reciprocal reduction of HCO\(_3\) was demonstrated in these animals so that the anionic sum remained relatively constant.

From a number of carefully conducted studies of the
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acid-base balance of the intestinal secretions we now know that the carbonic acid-bicarbonate buffering system is important in maintaining the hydrogen-ion concentration (219, 220, 322, 344, 413). The data of Robinson et al. (413) (Table 6) clearly demonstrate the components of the buffer system. These workers showed that the introduction of acid or alkaline solutions into the intestine results in an ion shift.

<table>
<thead>
<tr>
<th>Location of loop</th>
<th>No. of Expts.</th>
<th>pH 38°</th>
<th>HCO₃ mM</th>
<th>pCO₂ mm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunum</td>
<td>5</td>
<td>6.13</td>
<td>2.56</td>
<td>69.8</td>
</tr>
<tr>
<td>Jejunum</td>
<td>14</td>
<td>6.55</td>
<td>2.93</td>
<td>48.2</td>
</tr>
<tr>
<td>Jejunum</td>
<td>12</td>
<td>6.86</td>
<td>3.82</td>
<td>20.3</td>
</tr>
<tr>
<td>Jejunum</td>
<td>32</td>
<td>6.90</td>
<td>5.30</td>
<td>28.2</td>
</tr>
<tr>
<td>Jejunum</td>
<td>20</td>
<td>6.94</td>
<td>4.40</td>
<td>21.5</td>
</tr>
<tr>
<td>Ileum</td>
<td>15</td>
<td>7.14</td>
<td>10.07</td>
<td>28.8</td>
</tr>
<tr>
<td>Ileum</td>
<td>8</td>
<td>7.24</td>
<td>17.61</td>
<td>44.8</td>
</tr>
<tr>
<td>Ileum</td>
<td>18</td>
<td>6.91</td>
<td>10.39</td>
<td>45.6</td>
</tr>
<tr>
<td>Ileum</td>
<td>12</td>
<td>6.78</td>
<td>4.11</td>
<td>23.2</td>
</tr>
</tbody>
</table>

which brings the pH back to a “normal” for the particular part of the gut under consideration, and demonstrated that producing a systemic alkalosis with sodium bicarbonate caused a rise in the “normal” pH value for intestinal juice; on the other hand, an ammonium chloride acidosis did not affect the pH of the secretion. There is good evidence that the carbon dioxide of the intestinal juice is produced by secretory activity of the mucosa. McGee and Hastings (322) found that the average carbon dioxide tension of human jejunal juice is 100 mm. and concluded that active secretion was responsible. Robinson et al. (413) reported that when alkaline solutions were introduced into intestinal loops in the dog there was a rapid influx of carbon dioxide which produced a sharp pH drop. A compensating formation of bicarbonate followed, and the pH returned to a value which
was characteristic of the section of the intestine concerned.

Nitrogenous components of the succus entericus have been investigated to some extent. According to Florey et al. (155) the amount of "mucin" is extremely variable. Herrin (219) reported that pure jejunal juice from the dog contained 0.33 to 2.31% protein. Mosenthal (354) collected the secretions from six fistula dogs while following the total nitrogen intake and output of the animals, and came to the conclusion that the intestinal secretion contained up to 35% of the amount of nitrogen ingested.

Ammonia has been reported from the intestinal juice by several workers (219, 221, 236, 237). Pendleton and West (381) showed that urea passes quite readily from the blood to the lumen of the small intestine of the dog. Interestingly enough, Goudsmit (181) showed that urea could be removed from an animal by jejunal irrigation; nephrectomized dogs can be kept alive in this manner (416, 521). Clinically, a similar procedure has shown definite promise in the treatment of uremic patients (360, 368). Creatine and creatinine are secreted into the intestine in appreciable quantities (54). Ikeda (236) quantitatively determined the partition of non-protein nitrogen in the intestinal juice and reported the following: Total N—211 mg. %, ammonia N—24.2 mg. %, urea N—8.7 mg. %, creatine—1.62 mg. %, and creatinine—1.07 mg. %.

Lipid secretion in the small intestine was studied in fistula dogs by Sperry and Angevine (468). These workers found that much more was actually secreted than analyses of the feces indicated. Schoenheimer and Sperry (442) reported the presence of coprosterol, a metabolite of cholesterol utilization, in the feces of dogs with total bile fistulae; it was concluded that this substance arose from bacterial action on
cholesterol which was secreted by the intestinal mucosa.

A few other substances which may normally occur in the intestinal juice should be mentioned. There is some evidence that estrogens are secreted into the intestine (380). Heymann (224) showed that vitamin D is secreted into the small intestine. Another report, the implications of which are tantalizing, should be mentioned; in rats the rate of glucose absorption is reduced following adrenalectomy, but if the intestinal contents plus glucose of a normal rat are fed to an adrenalectomized animal the rate of glucose absorption is normal (199). Goodfriend et al. (178) reported the presence of a reticulocytogenic substance, discussed above in connection with Brunner's gland secretion, in the juice collected from the ileum of the pig. Reid (400, 401) showed that when ascorbic acid is administered intraperitoneally to the guinea pig considerable amounts of the vitamin may be found in the contents of the stomach and small intestine. Concentrations in the cecum and large intestine were lower, presumably due to bacterial destruction of the vitamin. Selye (451) has recently shown that riboflavin is secreted into the gastrointestinal tract. It is well known that the water soluble vitamins are excreted via the kidneys and the sweat glands. It is not surprising to find that some, or perhaps all, of them are normal constituents of the succus entericus. The implications of this possibility will be discussed further in a later portion of this paper.

The majority of the work on intestinal enzymes was done in the nineteenth century. A great deal of this information, along with some of the more recent work, is difficult to evaluate because of rather obvious faults in the experimental procedures. In most of the early work no effort was made to control pH; the precautions, if any, to exclude bacterial
enzymes were often inadequate. In other cases, no distinction was made between juices collected under distinctly abnormal conditions and the normal intestinal juices. The comprehensive work of Oppenheimer (367) should be consulted for a complete survey of work prior to 1936. A rather brief summarization of the knowledge of the intestinal enzymes will be offered in the following paragraphs.

It has been repeatedly demonstrated that the juice from an intestinal fistula digests starch, sucrose, maltose, lactose, poly- and di-peptides, and to a slight degree, fats. Some of the enzymes involved are known to be present in the cells of the mucosa. It is known that whole juice digests more rapidly than centrifuged juice (76, 273, 383). It has been demonstrated that if the particulate portion of the juice is mechanically sheared with glass, digestive activity is further increased (273, 383). This has been interpreted as an indication that some of the digestive enzymes are within desquamated cells contained in the juice.

Salaskin (431), Kutscher and Seeman (279), Waldschmidt-Leitz and Waldschmidt-Graser (512), Cajorí (76), and other workers have described a peptide-splitting enzyme or enzymes in the intestinal juice of dogs. Hamburger and Hekma (200), Bickel and Kanitz (50), and Owles (370) have described similar enzymes from man. Linderstrom-Lang (301) considered the peptidases to be typical endo-enzymes.

Schepowalnikow (434) first discovered enterokinase in the succus entericus of dogs in Pavlov’s laboratory. Hamburger and Hekma (200) demonstrated this enzyme in human intestinal juice. The few other workers who have looked for it since always found it present (26, 55, 143, 217, 291).

Lipase was first demonstrated in the succus by Boldyreff (56), who recognized that the lipolytic activity was weak.
Bickel and Kanitz (50) were unable to demonstrate lipase in the juice from a human ileal fistula. Owles (370) found weak activity in jejunal juice collected with a Miller-Abbott tube. Reale (395) was certain that lipase in intestinal juice was derived from the autolysis of desquamated cells. The evidence seems to indicate that lipase, if present, is found in only small amount in the succus entericus.

Dobroslawin (118) first reported amylolytic action in intestinal juice. Other workers who have looked for it in the juice have found it. Babkin (27) and Oppenheimer (367) give extensive references. Wohlgemuth's observation that ligation of the pancreatic ducts resulted in the disappearance of fecal amylase suggests that the intestine secretes relatively small amounts of this enzyme (527).

Invertase (=sucrase), lactase, and maltase have been reported by various workers from the intestinal juice. Bierry (51) found that centrifuging the juice or passing it through a Berkefield filter resulted in the loss of invertase activity. Cajori (76) and Rohmann and Nagano (420) investigated invertase activity of intestinal juice along with absorption of sugar, and concluded that sucrose was for the most part inverted during absorption. Florey, Wright, and Jennings (155) indicate that invertase is not found in any tissues other than the intestinal epithelium. Cajori (77) came to the conclusion that lactase is not secreted but is present in desquamated epithelial cells. Rohmann (419), Mendel (333), and other workers have reported slight maltase activity in the intestinal juice. Rohmann and Nagano (420) found that maltose was split by intestinal juice, but not as fast as it was absorbed by the mucosa. Bierry (51) freed the juice of cells and bacteria and found that maltase was still present. Alkaline phosphatase is a normal component of the succus (21, 274). It is
of interest to note the fact that phosphatase is present in
great amount in the intestinal mucosa; this has been used
in arguments favoring a phosphorylative mechanism of ab-
sorption. The writer would not presume to argue against the
validity of such a mechanism, since there is other evidence
to support the hypothesis. However, it should be pointed
out that the phosphatase in the mucosa may have no more
significance in phosphorylative absorption than amylase or
any other enzyme functioning in a hydrolytic capacity in the
gut. Mention should be made of the work of Rothstein and
Meier (426) which indicates that the cell “surface” phos-
phatases of yeasts are not concerned with carbohydrate me-
tabolism, but rather with the hydrolytic preparation of
phosphorylated compounds for absorption.

There is some evidence that diet may affect the quantita-
tive production of enzymes in the intestinal juice (60).

Some data on the amount of secretion is available. Boldy-
reff (56) described a periodic spontaneous secretion occur-
ring every one to two hours in the jejunum or ileum of strav-
ing dogs. This secretion was inhibited by food. A fistula 20 to
30 cm. long secreted as much as 2 ml. of juice in 20 to 30
minutes. From this Boldyreff calculated that the intestine
secreted about 30 ml. in such a period. Leper (295), Brynk
(70), and Komarov (266), corroborated Boldyreff’s work.
Babkin and Ishikawa (30) thought that the periodic secretion
was related to an increase in gut movement.

Local stimulation has a definite effect on secretion by the
intestinal mucosa. Thiry (489) described a flow of fluid into
the intestine when the mucosa was stimulated mechanically
with a sponge or catheter. In most subsequent work juices
collected from fistulae have been obtained with a catheter or
some modification of this method. Florey, Wright, and Jen-
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Nings (155) have pointed out that physiologists seem to accept such mechanically produced juice as true succus entericus.

Various chemical substances have been shown by Babkin (27) to stimulate secretion of juice by the intestinal mucosa. It should be said at this point that these effects of stimulation may have a very important bearing on the host-parasite relationship if we consider the parasite as a possible source of stimulation. There is probably some similarity in the physical effect produced by the contractions of a large tapeworm and that experimentally produced with a soft rubber catheter. A large nematode, Ascaris for example, might produce such an effect also. In the case of the smaller nematodes, hookworms for example, the mechanical irritation produced at the point of attachment could produce an alteration in intestinal secretion. The diarrhea produced early in a Trichinella infection and that produced by the small trichostrongyles in ruminants are probably gross indications of such irritation.

Among the chemicals used by Babkin (27) to obtain increased secretion were certain fatty acids, such as butyric. It is known that such materials are produced as end products in the metabolism of some intestinal helminths (74). Thus, the by-products of helminth metabolism may be stimuli for increased intestinal secretion.

If an intestinal parasite acts as a stimulus to cause increased intestinal secretion, what is the effect of the increased secretion upon the parasite? This question cannot be fully answered yet. It is of interest that Ackert and his coworkers (3, 5) have shown that at least a part of the host resistance to Ascaridia is due to some factor in duodenal mucus and that increased resistance may be correlated with an increase
in the number of goblet cells. Presumably, an intestinal helminth might also be affected favorably by materials secreted in the intestinal juice. Some evidence for such an effect will be brought forth later in this review.

The gastrointestinal hormones which have a role in the stimulation, and perhaps inhibition, of intestinal secretion have been discussed at length in a recent review (187) and will not be discussed in the present paper.

C. THE HEPATIC SECRETION

Before discussing the characteristics of the hepatic secretion, bile, it would seem appropriate to consider the anatomy of the biliary tract. Comparatively, there are some differences in the anatomy of the biliary tracts of various species. Most fish and amphibia, and all reptiles, have a gall-bladder. Gall-bladders occur irregularly among the birds; the chicken, goose, hawk, owl, and crow have gall-bladders, but the pigeon, parrot, humming bird, ostrich, cuckoo, and some cranes and falcons have none (467). Among the mammals, all carnivores, with the exception of whales, possess a gall-bladder, while some omnivorous and herbivorous animals do not (366). Animals with intermittent feeding habits are most apt to retain this structure, whereas herbivores with continuous feeding habits are most apt to lose it (179, 180). The horse, camel, deer (487), the peccary, sloth (Bradypus tridactylus), elephant, rhinoceros, some rodents (Rattus, Hyrax), and the cetaceans (whales, etc.) have no gall-bladder. In some cases there are considerable differences between closely related animals. In contrast to the rodents mentioned above, Mus musculus has a gall-bladder. According
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to Sobotka (467) the “pocket gopher” lacks a gall-bladder, while the “striped gopher” has one.
Oppel (366) has reviewed the comparative anatomy of the gall-bladder and bile ducts. There are variations in the number of extra-hepatic bile ducts and in the number of ducts emptying into the intestine. The orifice of the bile duct is usually very close to that of the pancreatic duct. However, there is some surprising variation in regard to

<table>
<thead>
<tr>
<th>Table 7. Amount of Bile Secreted by Various Species (467)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
</tr>
<tr>
<td>---------------</td>
</tr>
<tr>
<td>Dog</td>
</tr>
<tr>
<td>Cat</td>
</tr>
<tr>
<td>Sheep</td>
</tr>
<tr>
<td>Rabbit</td>
</tr>
<tr>
<td>Guinea Pig</td>
</tr>
<tr>
<td>Rat</td>
</tr>
<tr>
<td>Goose</td>
</tr>
<tr>
<td>Crow</td>
</tr>
</tbody>
</table>

this feature. In man, for example, both ducts usually enter the intestine through the papilla of Vater (306). In the rabbit the biliary opening is 35 to 40 centimeters above the opening of the pancreatic duct (106). It is obvious that the environment of a parasite of the rabbit inhabiting the region immediately adjacent to the bile duct opening would be somewhat different from that of a parasite living, for example, near the common biliary-pancreatic orifice in the intestine of the goat (467).

The amount of bile secreted daily by various animals has been investigated by several workers. Some of the reported data are presented in Table 7. There is some error in considering these data as valid indices of daily bile secretion in the intact animal in that the data were, in most cases,
from bladder fistula drainage. As will be brought out presently, the gall-bladder is definitely known to concentrate the bile by resorption of fluid. The extent of concentration is not well understood; thus, the volume of bile actually entering the intestine can be only roughly estimated at present.

The pH of the bile has been investigated by several workers. Some of the data are summarized in Table 8. It may be noted that gall-bladder bile tends to be more acid than hepatic bile. This is perhaps associated with the concentrating power of the gall-bladder. It has been observed (69, 357, 467) that all biles except sheep bile (1) become more alkaline on standing. This effect may be due to loss of CO₂, as suggested by Bronner (69); such is apparently the case with intestinal juices as previously mentioned. There is some evidence that the buffering capacity of bile may be rather important in neutralizing the acid chyme from the stomach. According to Neubauer (358) the bile requires an equal volume of N/10 acid to reduce the pH from the turning point of phenolphthalein (8.1) to that of methyl red (5.2). Schultz-Brauns (446, 447) studied the buffering power of bile from the gall-bladder and the liver. Two of his curves are reproduced in Figure 2. It is seen at once that the higher buffering capacity of gall-bladder bile may be a clue

<table>
<thead>
<tr>
<th>Species</th>
<th>Hepatic Bile</th>
<th>Gall-bladder Bile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Man</td>
<td>6.2–8.62</td>
<td>5.6–8.6</td>
</tr>
<tr>
<td>Dog</td>
<td>7.1–8.2</td>
<td>5.3–6.97</td>
</tr>
<tr>
<td>Cat</td>
<td>—</td>
<td>5.33</td>
</tr>
<tr>
<td>Sheep</td>
<td>—</td>
<td>5.98–6.73</td>
</tr>
<tr>
<td>Ox</td>
<td>—</td>
<td>6.74–7.47</td>
</tr>
<tr>
<td>Rabbit</td>
<td>—</td>
<td>6.4–6.7</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>7.7–7.9</td>
<td>7.2–9.1</td>
</tr>
</tbody>
</table>
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to one possible advantage to the animal possessing such a bladder.

The oxidation-reduction potential of the bile has not been investigated to any great extent. A few observations would indicate that negative Eh values prevail. It has been observed that, when methylene blue is fed to an animal, some

![Graph demonstrating the buffering power of the bile. L = hepatic bile; B = gall-bladder bile. (447)](image)

of the dye appears in the bile in the leuco form. Several investigators (68, 252, 262) found that fuchsin-S and indigo carmine injected intraglutally into human beings appeared in the bile in the colorless forms. The oxidation-reduction potential of biliary secretions should be further investigated; this physical factor may be of some importance in the physiology of parasites of the biliary tract.

The osmotic pressure of the bile is about equal to that of the blood. The freezing point depression for bile from several species is shown in Table 9. It should be noted that some of these values were obtained from fistula bile and some from gall-bladder bile. Brand (62) demonstrated that the osmotic pressure of bile is very stable and is not dependent on the
TABLE 9. OSMOTIC PRESSURE OF BILE

<table>
<thead>
<tr>
<th>Species</th>
<th>Freezing Point Depression</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ox</td>
<td>.54-.56</td>
<td>(124)</td>
</tr>
<tr>
<td>Ox</td>
<td>.54-.58</td>
<td>(334)</td>
</tr>
<tr>
<td>Ox</td>
<td>.53</td>
<td>(504)</td>
</tr>
<tr>
<td>Ox</td>
<td>.55-.60</td>
<td>(62)</td>
</tr>
<tr>
<td>Sheep</td>
<td>.59-.60</td>
<td>(334)</td>
</tr>
<tr>
<td>Hog</td>
<td>.45-.52</td>
<td>(334)</td>
</tr>
<tr>
<td>Man</td>
<td>.54-.627</td>
<td>(57, 63, 300, 334, 475, 478)</td>
</tr>
</tbody>
</table>

amount of total solids. Thus, during the concentration of the bile which occurs in the gall-bladder the effect of an increase in total solids is offset by a salt shift. Brand's data in Table 10 show that sodium chloride is involved. The ion shift during bile concentration has been verified in studies by other workers (85, 174, 392, 402).

The chemical constituents of the bile are probably better known than those of any other intestinal secretion. These substances include the bile acids (alkali salts), pigments, certain lipids, muco-protein, inorganic salts, carbon dioxide, ammonia, urea, and purine derivatives. Normally, the bile does not contain serum proteins in significant amount. Certain other non-physiological foreign substances may be excreted via the bile.

While no attempt can be made in this review to survey the monumental accumulation of literature relating to the bile

TABLE 10. DATA ILLUSTRATING THE SALT SHIFT ACCOMPANYING INCREASE OR DECREASE IN TOTAL SOLIDS OF THE BILE (62)

<table>
<thead>
<tr>
<th>Total solids (per cent)</th>
<th>NaCl (per cent)</th>
<th>ΔC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human (hepatic bile)</td>
<td>1.116</td>
<td>0.84</td>
</tr>
<tr>
<td>Human (hepatic bile)</td>
<td>4.08</td>
<td>0.572</td>
</tr>
<tr>
<td>Ox (gall bladder bile)</td>
<td>7.43</td>
<td>0.424</td>
</tr>
<tr>
<td>Ox (gall bladder bile)</td>
<td>9.16</td>
<td>0.85</td>
</tr>
</tbody>
</table>
acids and pigments, a few remarks on the former substances may be pertinent. Cholic acid is seemingly common to most mammalian species; the guinea pig and rabbit are exceptions in that they lack this bile constituent (163, 248, 278, 362, 437). Sobotka (467) has pointed out that these animals are distinguished by a relatively large bile flow which may be associated with qualitative peculiarities of the bile. Birds seem to possess a characteristic bile acid, cheno-desoxycholic, which is a predominant constituent (524, 533). Hog bile is unique in containing hyodesoxycholic acid (149). According to Hammarsten (201, 202, 204) sharks, polar bear, and seals have peculiarly characteristic bile acids. Amphibians also possesses unique bile acids (361, 457, 458).

A certain proportion of the bile acids are conjugated with taurine and/or glycine. The hog appears to be the only animal whose bile is devoid of taurine (467). Prevalence of glyco- over tauro-acids is common to man (467), cattle (44, 476), kangaroo (438), hippopotamus (205), and musk ox (203). This leaves the carnivorous animals in the tauro-group. The dog goes to the extreme in completely lacking glycine in the bile.

The inorganic constituents of the bile are present in approximately the concentrations to be expected in a transudate of the blood plasma. The ash content of hepatic bile indicates that the salt content of the bile fluctuates over a relatively narrow range (467). The inorganic constituents of the bile from man, dog, and rabbit are presented in Table 11. It may be seen that about half of the inorganic material is sodium chloride. The small amount of potassium present is in fairly constant proportion to the amount of sodium (175, 535). The calcium concentration is very near to that of blood (126, 510). It is of interest that in the gall-bladder the bile
calcium becomes more concentrated (127). This is evidently due to the lack of resorption of calcium by the bladder rather than an appreciable active secretion of calcium by the bladder (126). Discussing the calcium content of bile calls to mind the state of the hepatic bile ducts in older cattle perennially suffering from fascioliasis. In such animals the bile ducts are often heavily calcified. The writer has ruined the edges of several knives in collecting flukes from such livers. It would be of interest to determine the chemical nature of this insoluble material. It may well consist of the calcium salts of the fatty acids which *Fasciola* is known to produce as end products of its metabolism (66).

Other inorganic substances may be excreted from the body via the bile. These include cobalt (97, 98, 185), iron (185), antimony (223), arsenic (234), copper (448), zinc (348), and iodine (243, 244).

Of the lipids found in bile, cholesterol is probably the best known. This is undoubtedly due to its importance in the genesis of gall stones. This substance may be of some importance in helminth physiology since it has been indicated (495) that at least some invertebrates require cholesterol, but

### Table 11. The Inorganic Constituents of Hepatic Bile. Values are Milliequivalents per Liter

<table>
<thead>
<tr>
<th></th>
<th>Human (499)</th>
<th>Dog (402)</th>
<th>Rabbit (474)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCO₃⁻</td>
<td>—</td>
<td>34</td>
<td>46</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>96</td>
<td>64</td>
<td>82</td>
</tr>
<tr>
<td>Cholate⁻</td>
<td>—</td>
<td>76</td>
<td>—</td>
</tr>
<tr>
<td>Salts of other bile acids</td>
<td>—</td>
<td>26</td>
<td>—</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>Trace</td>
<td>—</td>
<td>4.4</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>7.3</td>
<td>Trace</td>
<td>2.5</td>
</tr>
<tr>
<td>Na⁺</td>
<td>—</td>
<td>174</td>
<td>161</td>
</tr>
<tr>
<td>K⁺</td>
<td>4.9</td>
<td>6.6</td>
<td>5.7</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>5.0</td>
<td>8.6</td>
<td>4.8</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>1.5</td>
<td>3.6</td>
<td>.5</td>
</tr>
</tbody>
</table>
are unable to synthesize it. The concentration of cholesterol in gall-bladder bile of several species is given in Table 12.

Phospholipid, according to Reinhold and Wilson (402), is quantitatively exceeded only by cholic acid as the most important bile constituent. Triglycerides, lecithin, glycerophosphate, fatty acids, and choline are known to be present in human bile (477). Of the fatty acids, acetic (119), propionic (119), stearic (61, 432), palmitic (61, 432), oleic (61, 432), and succinic (522) are known from human bile. Myristic acid has been recorded as occurring in ox bile (92). Neutral fats and lecithin are not present in the gall-bladder bile of the dog and pig (247).

Mucin is probably the most frequently reported nitrogenous constituent of the bile (467). As pointed out by Meyer (343), the term mucin is a rather loose one without a clear-cut biochemical basis. The term is used in the present review because our knowledge of the nature of the hexosamine-containing substances in the gastrointestinal secretions is rather scanty, with the possible exception of the gastric mucoid (186, 343). A protein resembling nucleoprotein has been reported from human bile (83). Urea has been reported as a constituent of the bile from dogs (315), oxen (167, 167a), and the shark (206); its concentration is parallel to that of

<table>
<thead>
<tr>
<th>Animal</th>
<th>Cholesterol content mg/%</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ox</td>
<td>30–70</td>
<td>(128)</td>
</tr>
<tr>
<td>Ox</td>
<td>70</td>
<td>(172)</td>
</tr>
<tr>
<td>Shark</td>
<td>0</td>
<td>(201)</td>
</tr>
<tr>
<td>Dog</td>
<td>110–140</td>
<td>(471)</td>
</tr>
<tr>
<td>Bear</td>
<td>Large amount</td>
<td>(201)</td>
</tr>
<tr>
<td>Hog</td>
<td>100–350</td>
<td>(123)</td>
</tr>
<tr>
<td>Rabbit</td>
<td>100–120</td>
<td>(372)</td>
</tr>
</tbody>
</table>
the total solids (388). Various amino acids are found in bile. Mueller (356) isolated glycine, lysine, and tyrosine from human bile and reported evidence for the presence of arginine and histidine. Yoshimura (534) reported the presence of amino acids, as well as uric acid, allantoin, creatine, creatinine, and ammonia, in fistula bile from the dog.

Hormones or substances with a related biological activity are, at least sometimes, constituents of the bile (80, 82, 190, 373, 378, 379, 380). Some of these substances have been isolated and identified. These include estrone (380), several metabolites of progesterone (378, 379), and alpha-estradiol (81). It would be of interest to ascertain with certainty the source of the estrogens and androgens found in the feces (120, 196, 298, 404, 461, 501). According to Marlow (313) bacteria are probably not concerned in producing the fecal androgens. There is some evidence that thyroxin is a bile constituent (137, 138, 243, 244, 290).

Certain vitamins may be present in the bile. Using pigeons and guinea pigs, Makimura (309) claimed to have shown that vitamins A, "B", C, and D are bile constituents. According to Makimura these substances are resorbed in the small intestine. When fed fresh bile rachitic guinea pigs showed a remission of symptoms indicating the presence of vitamin D. Ergosterol has been isolated from ox and dog bile (482). Cooper (100), attempting to study the vitamin content of ox bile, found that this material was so toxic when fed to the albino rat that it was not possible, by this type of experiment, to draw any conclusions as to its vitamin content.

Glucose has been found in the bile by various workers. Adlersberg and Roth (9) found no glucose in normal rabbit bile, but reported glycocholia as high as 580 milligrams per cent after the administration of phlorizin, a substance which
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blocks phosphorylative absorption in the kidney and the intestinal mucosa. On the other hand, Hirayama et al. (225) found 20 mg.% in rabbit bile and 58 to 88 mg.% in dog bile. Aszodi (24) reported 7 to 76 mg.% in fasting bile; according to Aszodi, glucose rapidly disappeared from the bile due to glycolysis. It is not clear whether bacteria were accounted responsible for this glycolytic activity, or the property of degrading glucose was attributed to the bile itself. The latter seems highly improbable to the writer for several reasons: (1) A complicated system of enzymes is necessary for glycolysis; (2) these enzymes are typically endoenzymes (intracellular); (3) none of the intermediates of glycolysis have been found in the bile (except lactic acid).

It seems more probable that the disappearance of glucose in the gall-bladder is due to resorption of sugar. The previously mentioned effect of phlorizin lends support to this hypothesis.

Because of its association with digestive activity, the enzymes found in bile have been studied by a number of workers. Amylase has been reported from the bile of the hog (58, 134, 254), human (58, 112, 414, 525, 532, 536), horse (134), sheep (254), ox (58, 254), and cat (254). After a study of the amylase in the bile of various species, Bonanno (58) concluded that there is more biliary amylase in herbivorous than in carnivorous animals. A biliary amylokinase, activating the pancreatic amylase, has been reported (303, 391).

Proteolytic enzymes have not been found in the bile, although a number of investigators have searched for them (58, 112, 134, 146, 536). Lipase occurs in the bile of several species (58, 134). The enzyme present in greatest amount in the bile is phosphatase (53, 113, 160, 259, 405, 406), which has been found in the bile of man, cat, and dog.
It would seem that Bonanno (58) was correct in saying that "the bile, from the enzymatic view-point, cannot be considered as a secretion distinguished by its digestive function," an opinion with which Sobotka (467) concurs.

D. The Pancreatic Secretion

Physiologically, the pancreas may be regarded as two organs in one. In the present discussion we shall consider only its exocrine secretion, the pancreatic juice.

The pancreas is a tubulo-alveolar gland which pours its secretion into the small intestine through the pancreatic duct or ducts. In the foregoing discussion of the bile some mention was made of the variations in the position of the duct orifice in different species. The arrangement of the duct system in mammals has been discussed by Huntington (235).

The alkalinity of the juice as reported by various workers varies over a wide range. Baylis and Starling (40) reported that pancreatic juice had an alkalinity equivalent to 0.1 N sodium carbonate. Other workers found titratable alkalinities varying from 0.05 to 0.16 N (117, 331, 352). The pH determinations of different investigators also show a wide variation, ranging from 7.06 to 10.21 (20, 25, 84, 102, 128, 156, 258, 472). Czubalski (102) showed that the alkalinity varied with the rate of secretion, obtaining pH values from 7.06 on slowly secreted juice to 8.64 on rapidly secreted juice. This finding corroborated earlier reports of this phenomenon (176, 527) and has subsequently been re-confirmed (32, 208). Gamble and McIver (170) showed that the chloride and bicarbonate concentrations of the juice vary inversely and suggested that this condition reflects the mechanism of
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pH control. Their suggestion is supported by Ball's data (32).

The inorganic constituents of the juice have been studied by a number of workers. Some of the determinations are presented in Table 13. It may be seen that the molar concentrations of inorganic materials suggest an osmotic pressure close to that of serum. That this is so has been amply confirmed. DeZilwa (117) and Pincussohn (385) reported

<table>
<thead>
<tr>
<th>Source</th>
<th>Na</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>Cl</th>
<th>HCO3</th>
<th>PO4</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>149</td>
<td>18.5</td>
<td>0.7</td>
<td>0.5</td>
<td>55</td>
<td>0.6</td>
<td>(439)</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>161</td>
<td>11.7</td>
<td>0.3</td>
<td>0.1</td>
<td>49</td>
<td>0.02</td>
<td>(166)</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>148</td>
<td>7.0</td>
<td>3.0</td>
<td></td>
<td>81</td>
<td>79</td>
<td></td>
<td>(170)</td>
</tr>
<tr>
<td>Dog</td>
<td>144*</td>
<td>4.3</td>
<td></td>
<td></td>
<td>50</td>
<td>59</td>
<td>0.13</td>
<td>(209)</td>
</tr>
<tr>
<td>Dog</td>
<td>148</td>
<td></td>
<td>1.7</td>
<td>0.4</td>
<td>120</td>
<td>41**</td>
<td>0.3</td>
<td>(34)</td>
</tr>
<tr>
<td>Man</td>
<td>144</td>
<td>6.4</td>
<td></td>
<td></td>
<td>51</td>
<td></td>
<td></td>
<td>(449)</td>
</tr>
<tr>
<td>Man</td>
<td>132</td>
<td>2.5</td>
<td>Trace</td>
<td>Trace</td>
<td>129</td>
<td>2.3</td>
<td></td>
<td>(527)</td>
</tr>
</tbody>
</table>

* Reported as total base.
** As CO2.

that the freezing point depression of juice from the dog averaged $-0.610^\circ$ C. Hartmann and Elman (209) and Ball (33) reported that the freezing point depressions of blood and pancreatic juice from the dog are identical. Glassner (176) and Wohlgemuth (527) reported that the freezing point depression of human juice averaged $-0.465^\circ$ C. On the other hand, Luckhardt et al. (305) reported a freezing point depression of $-0.625^\circ$ C. for juice from man.

It has been recognized for some time that the sodium, potassium, and bicarbonate of the juice probably come directly from the blood. Ball (33) showed that intravenous injection of these ions caused changes in their concentrations in the pancreatic secretion corresponding to the concentration changes in the blood. His findings are supported by
Oldfeldt's studies (364) and by tracer experiments with radioactive sodium (348) and C\textsuperscript{14}-labeled bicarbonate (35). On the other hand intravenous injection of calcium, magnesium, or phosphate showed little effect on the concentration of these ions in the juice. Ball (33) noted that calcium, magnesium, and phosphate are found in the juice in concentrations one-twentieth to one-fourth those in blood serum and concluded that the pancreas must be only slightly permeable to these ions.

Other inorganic substances, such as cobalt (454) and zinc (349), may be present in the pancreatic secretion.

Several proteins occur in the pancreatic juice. Albumins and globulins have been found in juice from man (135, 176, 527). Hartmann and Elman (209) report an albumin/globulin ratio of less than \( \frac{1}{2} \). According to deZilwa (117) nucleoprotein is present in pancreatic secretion of the dog. Hartmann and Elman (209) reported that dog juice contained 0.8 to 1.9% protein (av. 1.2%). Komarov et al. (268) found 110 to 341 mg. of protein nitrogen per 100 ml. in juice from dogs.

Chemical analyses of the organic constituents of pancreatic juice are not plentiful. Lactic acid is present in dog juice in concentration somewhat lower than in the blood, and urea is present in about the same concentration as in the blood (209). Ball and Johnson (34) reported the total nitrogen of dog juice as 79 to 879 mg. per cent. Williams et al. (523) found that a B-complex vitamin, inositol, was present in pancreatic amylase preparations. In a later paper (281) it was shown that inositol functions as a coenzyme for alpha-amylase. It would be of interest to determine the presence or absence of other vitamins in pancreatic juice.

The enzymes of the pancreatic juice have been studied extensively. In contrast to the bile, we may say that pan-
creatic juice is a secretion distinguished for its high content of digestive enzymes.

It has long been known that pancreatic juice possesses the property of digesting proteins and for a long time it was thought that this was due to the presence of a single enzyme, trypsin. About 1900 Schepowalnikow (434) showed that the tryptic enzyme was activated by a substance in the intestinal juice. This was called enterokinase, "a ferment of ferments" (376). The mechanism of this activation was the subject of much controversy in subsequent years and there were numerous contradictory experimental results (359). These led to the conclusion that more than one protease was present in pancreatic secretion, and culminated in the isolation of three crystalline enzymes: trypsin (277), chymotrypsin (276), and carboxypeptidase (19). The first two are mainly responsible for the proteinase activity of the juice, while carboxypeptidase acts on the amide linkages of peptides produced in protein degradation. There may be additional peptidases in the juice, but these are not yet adequately characterized (49).

In addition, lipase, amylase, and maltase are always present in the pure pancreatic juice of man, dog, and cat (29, 230, 512).

It has been shown that the quantitative enzyme makeup of the pancreatic juice shows some adaptation to the composition of the diet (188, 189). The effects of deficiencies produced by deprivation of pancreatic enzymes have been discussed by Handelsman (207).

The amount (in grams) of pancreatic juice secreted per kilogram of body weight per day was estimated to be as follows: horse, 16.8; ox, 14.4; sheep, 12.0; pig, 7.2; dog, 2.4 (94). Assuming a specific gravity of about 1.01 (129) it may
be calculated that the daily secretion in a thousand pound horse is more than seven liters; in an ox of the same weight the secretion would be something over six liters.

Although we shall not discuss the control of pancreatic secretion, it is well to point out that there is a small continuous secretion of juice in most species (29, 30, 493). In the rabbit there is a copious continuous secretion which is little affected by feeding or digestive activity (38, 39).
III. THE EXOCRINO-ENTERIC CIRCULATION

The above term has been selected to describe the flow of materials, by diffusion or active transfer, into the intestinal lumen and the subsequent intestinal resorption of these materials. For the parasitologist interested in the physiology of non-tissue feeding helminths, such as Ascaris, the cestodes, or the acanthocephalans, the exocrino-enteric circulation assumes great importance. Substances which take part in such a circulation become available to a parasite in the intestinal lumen. Thus, a parasite may remove substances which the unparasitized host would resorb for utilization. If some hypothetical substance undergoing exocrino-enteric circulation is an important micronutrient, i.e., a vitamin, and the host is in a “borderline” state of nutrition, removal of some of this substance by intestinal worms could precipitate a deficiency syndrome.

There is a good deal of evidence to indicate that an exocrino-enteric circulation actually occurs. Before presenting the data for consideration a few comments of a general nature seem pertinent. The presence and concentration of any given substance in the lumen of the intestine would be conditioned by a number of factors: (1) The rate of entrance, whether in the chyme from the stomach, in the secretions of the intestine, and/or by diffusion from the tissues of the mucosa; (2) the rate of breakdown or metabolic transformation by the action of microorganisms or by the enzymes of the intestinal secretions; (3) the rate of absorption (active transfer) and/or diffusion out of the intestine; (4) the rate at which materials are propelled through the intestinal tract;
The concentration of other substances in the tissues or in the lumen of the intestine. Obviously, there is some interdependence of these factors. The second factor would not seem to be important in the case of inorganic ions, but it must be remembered that chemical combination of such ions as sodium may remove them from consideration as ions. From these considerations it is apparent that the net absorption or excretion of a given substance is not a simple phenomenon. Some of the evidence from which the foregoing statements have been adduced may become apparent in the paragraphs to follow.

The so-called entero-hepatic circulation of the bile acids is now accepted as an established phenomenon. The evidence for such a circulation has been reviewed by Sobotka (467) and Josephson (249). According to the accepted concept, the bile acids secreted by the liver are resorbed in the intestine.

The site of bile acid resorption may be of importance to an intestinal helminth. According to Tappeiner (484) bile salt resorption occurs chiefly in the ileum; none of the acids are resorbed in the duodenum and only glycocholic acid in the jejunum. Frolicher (165) verified Tappeiner’s work on the site of resorption. Thus, a parasite in the lower part of the small intestine may be exposed to significant concentrations of bile salts. However, these are not necessarily in the same ratio of concentration, i.e. glycine/taurine, as in the bile. Furthermore, we may question whether the site of bile acid resorption is the same in all species. This has not been determined in enough species to allow a generalization.

The actual amount of bile salts passing through the small intestine is astounding. Whipple and Smith (520) showed that a ten kilogram dog keeps seven to eight grams of bile
salts in circulation by resorption and re-excretion, and the circulation period for this amount is eight to sixteen hours. In recent years this idea has been extended to embrace substances other than the bile acids. Cantarow and his associates (82) after an extended study of the hepatic inactivation of estrogenic substances have postulated that these compounds undergo entero-hepatic circulation. These workers found that 24 to 48 hours after the intravenous injection of alphaestradiol a large amount of the estrogen was present in gallbladder bile. Nothing has been reported on the site of resorption of such substances.

Some vitamins may undergo entero-hepatic circulation. Fecal excretion values of choline are extremely low (332). Since this is known to be a bile constituent it suggests the possibility of resorption of this B vitamin. As noted in the discussion of bile, vitamins A, "B," C and D have been reported by Makimura (309) to be normal bile constituents. According to this investigator, these vitamins are resorbed in the intestine, thus undergoing an entero-hepatic circulation. This problem would bear further investigation.

The entero-hepatic cycle may be of some importance in the chemotherapy of intestinal helminthiases. As a case in point, Culbertson (101) has shown that atabrine is highly effective against *Hymenolepis nana* in mice. This drug undergoes enterohepatic circulation which may aid in maintaining anthelmintic concentrations in the gut.

There is also evidence of an entero-enteric circulation. Herrin (219) studied the effect of loss of the succus entericus on fistula dogs. He reported that continued loss of the juice caused marked changes in the chemical composition of the secretion. There were decreases in the chlorides and fixed base, a great increase in bicarbonate, and the appearance
of large amounts of ammonia. Herrin found that the serum of his experimental dogs showed average decreases of 26% in the chloride concentration, 20% in the bicarbonate concentration, and 10% in fixed base. There was a marked rise in the serum concentrations of calcium, phosphorus, and protein. A 30% to 40% reduction in the plasma volume was recorded with a great increase in blood viscosity. Anoxia was observed and death occurred. Since Herrin's animals were given sufficient water it is plain that resorption of water was not the primary deficiency induced by loss of the intestinal juice. This study alone furnishes strong evidence of an entero-enteric circulation.

Recently, Visscher and his associates (508, 509) have made some very significant observations on the movement of water and ions between the intestinal lumen and the blood. These workers utilized the radio-isotopes of sodium and chlorine and the heavy isotope of hydrogen, deuterium. By these methods it was possible to avoid the use of non-physiological substances or non-physiological amounts of the substance under investigation. Visscher et al. reached the following conclusions: Forces other than simple physical diffusion account for most of the movement of water between the intestinal lumen and the blood. No movement can occur without a driving force, and it is therefore apparent that movement of water across the intestinal epithelium is brought about by some sort of forced flow. Furthermore, there is a forced flow of fluid across the intestinal epithelium in both directions simultaneously; differences in the solute content of the water in the two streams and the relative rates of the streams determine the direction and magnitude of the net transport. At this point, we may again call attention to Osterhout's recent demonstration (369) that a cell may take
up water at one spot on its surface and simultaneously expel water at another spot. Osterhout postulated that this may be due to non-uniformity of intracellular osmotic pressure.

So far we have mentioned only water and inorganic ions in connection with entero-enteric circulation, but there is some evidence that other substances may take part in the process. Wright et al. (531) actually observed the resorption in the jejunum of duodenal secretion. Mosenthal (354) studied the amount of nitrogenous material in the succus entericus. Using six fistula dogs, Mosenthal collected the intestinal secretion while following the total nitrogen intake and output. It was found that the intestinal secretion contained up to 35\% of the amount of nitrogen ingested. Since the fecal nitrogen was usually about 10\% of the amount of nitrogen intake, Mosenthal reasoned that at least 60\% of the nitrogen secreted into the lumen was reabsorbed.

Lipid secretion in the small intestine of fistula dogs was studied by Sperry and Angevine (468). It was found that there was a much larger excretion of lipids in the small intestine than was indicated by analyses of the feces. It was concluded that considerable amounts of the lipids secreted in the small gut were reabsorbed. This conclusion is supported by other experiments in Schoenheimer’s laboratory. Using fats labeled with heavy hydrogen it was found that 65\% to 70\% of the dietary fatty acids were absorbed in the absence of bile and that the increase of fecal fat observed in animals deprived of their own bile was due to faulty resorption of secreted fats (452).

Some vitamins may undergo entero-enteric circulation. According to McGoogan (324) it is now well established that a polynoeritis which is relieved by thiamine administration is sometimes observed following the pernicious vomiting of
pregnancy. This implies that there may be a “leakage” of thiamine from the mucosa. It is reasonable to assume that this vitamin would normally be reabsorbed. Chandler, Read, and Nicholas (89) have recently furnished additional evidence for the exocrinoenteric circulation of thiamine. The vitamins previously discussed as components of the succus (ascorbic acid, riboflavin, vitamin D, and B₁₂) may well be involved in entero-enteric circulation.

That some entero-enteric circulation may occur independently of the digestive activities is indicated in the reports that goblet cell secretion is autonomous and not related to digestive activity (90) and that gastric mucin secretion is independent of acid and pepsin secretion (269). Babkin (29), reviewing the literature on gastric secretion, noted that there is a continuous secretion of juice in the abomasum of ruminants.

A pancreato-enteric circulation also occurs. Strong evidence for this circulation is furnished by studies on dogs deprived of the external secretion of the pancreas (136, 170, 209, 319). Complete pancreatic fistulae with continuous loss of the secretion leads to death in five to eight days. The mechanism leading to a fatal ending is not related to the digestive function of the pancreatic secretion, but rather to profound alteration of the acid-base economy. This phenomenon has been discussed in detail by Handelsmann (207). In some animals it is obvious that there must be a resorption of pancreatic fluid. The rabbit, for example, secretes pancreatic juice at the same rate all the time (38, 39). If there were no resorption of juice the feces of this animal would be of a quite different consistency than they are.

Inositol, which has been mentioned as a constituent of pancreatic juice (523), is probably resorbed in the intestine.
The presence or resorption of other vitamins in the pancreatic secretion has apparently not been studied.

From the above discussion we may construct a tentative picture of an exocrin-enteric circulation. Substances may be secreted (sensus latus) into the intestine and be resorbed. These substances include inorganic ions, lipids, nitrogen-containing compounds, and at least some vitamins. Glucose is conspicuous by its absence. We may theorize that this might indicate that substances which are normally absorbed by mechanisms of phosphorylation probably do not enjoy an exocrin-enteric circulation.
IV. THE PARAMUCOSAL LUMEN

The above term has been chosen by the writer to designate the portion of the lumen of the intestine which is immediately adjacent to the mucosa. There is good evidence to support the idea that the physico-chemical conditions adjacent to the mucosa are different from conditions in the center of the lumen. In the following discussion the writer will attempt to furnish support for the concept of such a physiological stratum.

It has been recognized for some years that the pH near the mucosa is probably not the same nor subject to the same degree of fluctuation as the central part of the lumen. Kofoid et al. (264, 265) reported definite differences in the pH of the intestinal contents and the intestinal wall of the rat. Robinson (409) passed isotonic calcium chloride solutions through jejunal and ileal loops in dogs and reported that each part of the intestine tended to establish its own characteristic pH irrespective of the pH of the original solution. He suggested that there is a zone at the surface of the mucosa where the reaction is stabilized and that this value is not necessarily given by measurement of the pH of the intestinal contents at that point. Ball measured the pH near the mucosa in the rat with a capillary electrode (36, 37). Robinson et al. (413) corroborated the earlier work (409) and examined the factors producing the characteristic pH. Their data indicated that the progressive increase in pH in the small gut is due to a combined decrease in carbon dioxide tension and an increase in bicarbonate concentration of the intestinal secretion. Buecher et al. (71) confirmed this finding.
and showed that in man there were decreases in the chloride roughly equivalent to the bicarbonate increase.

If we consider that the osmotic characteristics of intestinal juice probably represent those of the paramucosal lumen, we may conclude that the osmotic pressure of the paramucosal lumen is probably close to that of blood serum. The evidence for the isotonicity of the secretions has already been discussed on page 3.

The gases in the paramucosal lumen are probably quantitatively different from those in the central lumen. Roger's study on the oxygen tensions adjacent to the mucosa in the intestines of rats and sheep has already been mentioned (418). His findings in the case of sheep are of particular interest. He reported oxygen tensions of 4.0 to 12.7 mm. Hg near the mucosa. On the other hand, previous analyses of the contents of the lumen have indicated that no oxygen is present in the small intestine of the sheep (486). The difference in the gaseous content of the para-mucosal and the central lumen is indicated by the variance in these results.

The probability of a higher oxygen tension near the mucosa has been indicated in previous reviews (64, 65, 228). However, no data are available to indicate possible differences in the concentrations of other gases in the paramucosal and the central lumen.

The higher oxygen tension of the para-mucosal lumen suggests that there are differences in the oxidation-reduction potentials of the central and the para-mucosal lumen. It would be difficult to reconcile an oxidation-reduction potential of —100 mv., the value reported for the contents of the small intestine of the rat (47), with an oxygen tension of about 8 to 30 mm. Hg in the para-mucosal lumen. The oxidation-reduction potential of this region may actually be close to zero.
It has been indicated that there are various organic and inorganic substances which are characteristic constituents of the intestinal secretion (page 18 ff.). The concentration of these substances falls within certain normal limits. It is obvious that as these materials are secreted they will tend to diffuse into the contents of the intestine. It is probable that some of the organic materials are here altered by the action of microorganisms. Thus, we might assume that the concentrations of these materials will not be the same in the para-mucosal and in the central lumen. There is some strong evidence that some intestinal helminths which do not feed on the host tissues obtain a significant portion of their nourishment from the host stores (see page 70). Thus, concentration factors may be of great importance.

The recent evidence of an autonomous secretion independent of digestive activity (90,269) lends further support to the concept of a space near the mucosa, a physiological stratum, where conditions may be more like those of the intercellular spaces of the host than might be surmised from study of the intestinal contents.
V. THE INTESTINAL FLORA AND HELMINTH PARASITES

DURING the past five decades much attention has been focused on the relation of intestinal microorganisms to the well-being of the host. Yet, despite a voluminous literature on the species and distribution of the bacteria found in the gut of man and many other warm-blooded animals, the relationships are still quite imperfectly known. It has become increasingly evident that the microbial ecology of the intestine is complex.

In recent years biologists have come to regard intestinal bacteria as one source of certain necessary trace substances, vitamins. It is now well recognized that intestinal synthesis of vitamins may be actually indispensable to the host, particularly if the diet is not properly balanced. Early workers considered the bacteria inhabiting the intestine to be important mainly by virtue of the supposed harm wrought upon the host through the production of the toxic products of putrefactive metabolism. In recent years evidence has accumulated to show that intestinal bacteria are important in supplying essential nutritional factors. On the other hand, the production of toxic materials in significant amount, in the absence of disease, is still somewhat questionable. Najjar and Barrett (356a), reviewing vitamin synthesis by intestinal bacteria, remarked, “It is . . . comforting to know that we can at least rely to some extent on this mechanism to supplement the average American diet should the ever persistent vitamin vendor fail in his benevolent task.”

Concomitantly with the development of this concept, a
A growing body of evidence has shown that there are numerous influences upon the types, numbers, and chemical activities of intestinal bacteria. These influences include diet, pH, Eh, the animal species involved, the digestive activity, and possibly other factors such as surface tension, mineral concentrations, natural metabolic, antagonists, synergisms, and age and sex of the host animal. Obviously, there is a high probability that the bacteria may affect, as well as be affected by, such things as pH, Eh, etc. All these factors, of course, make for a rather delicate balance of intra-intestinal life.

The location in the intestinal tract of the greatest degree of vitamin synthesis differs in various animals. In cattle and other ruminants most of the vitamin synthesis occurs in the rumen. This means that the materials which are synthesized pass through a major portion of the alimentary tract before being lost to the host, and thus are available in the regions particularly specialized for absorption. It is interesting that the study of rumen synthesis was initiated by Theiler and his co-workers (488a), familiar to parasitologists through other researches. Detailed information on rumen synthesis is provided in several excellent reviews (211, 271, 463, 488).

In rats, on the other hand, and probably in man, the greatest degree of vitamin synthesis apparently occurs in the cecum, which is located past the regions best fitted for absorption of food materials. Nevertheless, there is abundant evidence that there is enough absorption from the cecum to aid materially the non-ruminant in satisfying his vitamin requirements. Taylor et al. (487a), using normal and cecectomized animals, found that the cecum of the rat has a significant role in vitamin synthesis when the animal is maintained on an inadequate diet. Day, et al. (110) produced evidence that the cecum may be the main site of synthesis.
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and absorption of vitamin K. A highly significant study by Mitchell and Isbell (347) shed light on the degree of vitamin synthesis in the cecum of the rat. These workers attempted to determine the site of absorption and the availability of certain B complex vitamins. They studied the synthesis of nicotinic acid, pantothenic acid, thiamine, riboflavin, pyridoxine, biotin, inositol, and pteroylglutamic acid (folic acid) in rats on a lean beef ration, with and without lactose. After removal of the ceca of the experimental rats, the cecal contents were centrifuged and the vitamin content of the supernatant fluids and the residues were determined. They reported that inositol, thiamine, riboflavin, nicotinic acid, and pantothenic acid were tied up in the cells to a considerable extent whereas the other members of the B-complex studied would readily diffuse from the cells into the medium. To obtain information on the site of absorption, sections of the walls of the stomach, upper and lower small intestine, cecum, and colon were ground, extracted with boiling water, and analyzed for biotin, pantothenic acid, and pteroylglutamic acid. It was found that the greatest absorption of these B vitamins took place in the cecum. From their data Mitchell and Isbell calculated the approximate percentages of the dietary intake of B vitamins which were supplied by intestinal synthesis: thiamine, 8% to 18%; riboflavin, 5 to 19%; nicotinic acid 1.8% to 3.5%; pantothenic acid 11% to 58%; pyridoxine, 130% to 230%; biotin 230% to 430%; inositol .08% to .1%; and pteroylglutamic acid, 67% to 71%. Obviously certain vitamins are more available to the animal than others. This fact is borne out by the earlier studies of Guerrant et al. (191, 192, 194, 195) which showed that a large proportion of synthesized riboflavin and thiamin is passed out of the body before it can be absorbed. Denko et al. (114)
have indicated that the same thing is also true in man. Detailed reviews of the evidence in favor of the view that the cecum is the main site of synthesis are available (104a, 139, 241, 356a).

Because vitamin synthesis in the cecum of non-ruminants is quantitatively greater than in other parts of the alimentary tract, nutritionists have tended to place great emphasis on this segment of the alimentary tract. Such emphasis is to be expected since these workers are mainly concerned with the vitamin needs of the host. Some papers may be found in the literature which leave the distinct impression that the cecum is the sole site of vitamin synthesis. Such is not the case. There is evidence to indicate that, in non-ruminants, synthesis of vitamins occurs in segments of the gut other than the cecum. However, the quantities of the needed factors produced in, for example, the small intestine are such that a substantial contribution from this source to the vitamin economy of the host is not probable. On the other hand, the amount of a given vitamin produced by bacterial synthesis in the small intestine may represent a significant portion of that required by a helminth parasite.

Evidence in support of this view has been provided by a number of recent workers. Porter and Rettger (389) reported that, contrary to prevailing opinion, the stomach and small intestine of the albino rat under most dietary conditions contain appreciable numbers of viable bacteria and yeasts. Johansson et al. (242) showed that significant numbers of bacteria and yeasts are present in the small intestine of the hen. It has been shown that the bacteria in the small intestine of the mouse may play a role in vitamin synthesis (168, 169). It is of interest to note that the presence of an intestinal helminth may change intra-intestinal conditions to
such an extent that increased numbers of microorganisms are found in the small intestine. This may be indicated in Becker's report (42, 42a) that *Escherichia coli* is found more often in the stomachs of humans harboring *Dibothriocephalus* than in the stomachs of unparasitized individuals. Najjar and Barrett (356a) noted that the discharge from an ileostomy fistula in a human infant contained a measurable amount of thiamin which was abolished by oral administration of sulfasuxidine. These workers remarked that "... apparently thiamine synthesis is not confined to the large gut." The results of Day et al. (110) indicated that the cecum was not the only location of vitamin K synthesis in the rat. Cecectomized rats on a ration deficient in vitamin K grew well and evidenced no K deficiency unless 1% sulfasuxidine was incorporated in the ration; rats with intact ceca on the same ration with sulfasuxidine showed no K deficiency. McGregor et al. (325), studying biotin balance in the albino rat, found that analyses of the biotin in the lumen of the small intestine indicated bacterial synthesis or excretion of this vitamin.

The types of microorganisms which are responsible for intestinal vitamin synthesis are not clearly defined. It has been suggested that yeasts are prominent in synthesis (195). It has been shown that various B vitamins are synthesized *in vitro* by several species of bacteria known to occur in the gastrointestinal tract. These vitamins include riboflavin (75, 466, 491), nicotinic acid (75, 491), pyridoxine (491), pantothenic acid (491), biotin (75, 491), pteroyloglutamic acid (491), inositol (491), thiamin (75, 397, 398, 407, 408, 435, 436, 481, 491, 514). However, culturing of microorganisms *in vitro* imposes conditions which are certainly quite different from those existing *in vivo*. It seems likely that several species are involved in vitamin production as well as vitamin
destruction and that a dynamic equilibrium exists between the various species inhabiting the gut (45, 104a, 241, 356a). These data may serve to support the thesis that bacterial synthesis may be of some importance in satisfying the vitamin requirements of intestinal worms.

In planning experiments designed to ascertain the vitamin requirements of intestinal parasites it is desirable to survey factors which may influence bacterial synthesis and vitamin availability. Such a survey brings to light some rather complicated features.

Since the days of Metchnikoff (335), diet has been known to affect the bacterial population of the intestine. A number of early workers reported that altering the carbohydrate of the diet had an effect on the intestinal flora (222, 402a, 403). In animals on a high carbohydrate diet, the intestinal flora was predominantly an acidophilic non-proteolyzing type; however, on a diet high in protein there was a change to a strongly proteolyzing flora (222).

It has long been known that specific carbohydrates have different effects on the growth of bacteria. As might be expected, bacterial vitamin synthesis is affected also. It was recognized several years ago that increased "vitamin B" synthesis occurred when the dietary carbohydrate was rice starch (423, 424), dextrin-containing starch (162), or potato starch (270, 272). Guerrant and Dutcher (192, 193) showed that feeding dextrinized corn starch as the only carbohydrate in the diet of the rat increased the synthesis of thiamine and riboflavin enough to lower the dietary requirements for these two factors. These workers showed that poor synthesis of the above-mentioned factors was obtained when commercial starch, sucrose, glucose, or lactose were fed. Morgan et al. (353) studied the relation between type of dietary car-
bohydrate and deficiencies of riboflavin, pyridoxine, and pantothenic acid in rats. Lactose favored synthesis of riboflavin and pyridoxine; corn starch favored synthesis of pantothenic acid, and sucrose favored synthesis of none of these three members of the B-complex. Elvehjem (139) reported that guinea pigs thrived on a synthetic diet containing dextrin when 15% yeast was added. If the dextrin was replaced by sucrose the animals were not nutritionally supported. It has been reported that addition of lactose to the diet stimulates synthesis of pteroylglutamic acid and niacin in the intestine of the rat (140).

Dietary factors other than carbohydrates are also important. According to Wegner et al. (513) increased nitrogen in the feed suppressed synthesis of riboflavin in the rumen. The inclusion of lard in the diet of rats promoted thiamin synthesis (518). The work of Guerrant and Dutcher (193) indicated that the amount of roughage in the diet may have an effect on bacterial synthesis.

The dietary vitamins also exert some influence on intestinal synthesis. Martin (316) reported that when the B-complex of a synthetic ration was supplied as riboflavin, thiamin, nicotine acid, pyridoxine, choline, and Ca-pantothenate, mice grew well. If para-aminobenzoic acid was included in the ration, the mice developed a deficiency syndrome like that seen in pantothenic acid deficiency; this was corrected by adding inositol to the diet. Conversely, if inositol was incorporated in the diet, a para-aminobenzoic acid deficiency developed. Martin’s data indicated that inositol may stimulate proliferation of pantothenate-destroying organisms, and that para-aminobenzoic acid may inhibit them. Woolley (529) reported interesting evidence of an interrelationship in the vitamin economy of the gut. He found that an
inositol-free synthetic ration supported good growth of mice. However, in the absence of pantothenic acid the mice developed an alopecia typical of inositol deficiency. The inositol-synthesizing mice yielded mixed cultures of bacteria from their droppings which were able to form considerable amounts of inositol. Mixed cultures from the deficient mice synthesized very little inositol. Thus, it would seem that an interplay of factors may be present. Moore (351) reviewed the knowledge of the interrelationships of vitamins and indicated that a deficiency of one factor may change the requirement for another. This complementary effect is not well understood, but, considering the role in enzyme systems which some of the vitamins are now known to play, it is not surprising.

In 1940 Marshall et al. (314) reported the bacteriostatic effect of the poorly absorbed drug, sulfaguanidine, on intestinal bacteria. This provided a new approach to the study of intestinal synthesis. Within a short time it was found that inclusion of certain sulfonamides in the diets of rats resulted in deficiencies corrected by para-aminobenzoic acid, biotin, pteroyloglutamic (folic) acid, and vitamin K. It would seem that the incorporation of sulfonamides in the diet of the host with a consequent reduction of intestinal synthesis might be useful in the study of the nutritional requirements of an intestinal helminth. Some work along these lines has been carried on in this laboratory (89). While sulfonamides in the diet may have some use as a tool, there are some pitfalls in this approach:

1. It must be demonstrated that the drug has no harmful effect on the host or the helminth parasite.
2. It is not probable that intestinal synthesis is completely abolished. The actual amount of a given factor required
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by a parasite may be so small that it is impossible to produce a deficiency.

(3) The parasite may obtain enough to satisfy its requirement from a source other than the diet or intestinal synthesis, i.e., from the secretions of the host.

(4) A vitamin imbalance may result. Olcese et al. (363) showed that feeding 1% sulfasuxidine to rabbits on a deficient diet actually produced an increase in the amount of riboflavin in the feces. Moore et al. (350) showed that the sulfa drugs, and streptomycin as well, enhance the intestinal synthesis of pteroyloglutamic acid in chickens. This effect was explained as being due to a modification of the intestinal flora resulting in the suppression of those organisms which have a pteroyloglutamic acid-destructive action or which usually inhibit pteroyloglutamic acid synthesis.

(5) Other micronutrients in the diet may tend to nullify the effect of the drug. For example, it was found that in the sulfasuxidine-induced vitamin K deficiency of the rat, addition of folic acid and biotin to the diet resulted in some remission of K-deficiency symptoms (516). Krehl et al. (275) reported that dogs receiving a synthetic diet containing six of the B vitamins, excluding pteroyloglutamic acid, grew well even when 4% sulfasuxidine was included in the diet. However, during nicotinic acid deficiency the dogs showed a requirement for dietary pteroyloglutamic acid. In experiments on the nutrition of pigs, it was found that the addition of inositol to a basic diet containing sulfathalidine alleviated the symptoms of the biotin deficiency induced by such a diet (302). These various observations have been interpreted to indicate stimulation of the growth of
certain organisms which are probably not affected by the sulfa drug.

Another method of studying the vitamin requirements of parasites in vivo may be in the use of the antimetabolites, vitamin analogues (150, 515). Again, certain difficulties may arise when we realize that the intestinal bacteria will probably be markedly affected by antimetabolites.

Coprophagy may constitute an error in some host feeding experiments. In 1928, Fridericia et al. (162) and Roscoe (423) independently published papers describing spontaneous recoveries of rats which had been depleted on a vitamin B-complex deficient ration. Fridericia et al. (162) called this unusual process refection. It was concluded that the spontaneous recoveries observed in their work were due to consumption of reected feces, since the protective effect could be transmitted from animal to animal by feeding feces from reected animals. Furthermore, it has been shown by Almquist and Stokstad (12), and Lamoreux and Schumacher (280) that there is considerable synthesis of vitamin K, riboflavin, and biotin in the feces of rats and chickens after expulsion from the body. Recently, Kennard and Chamberlin (255) have suggested that it may be desirable to allow floor-litter to accumulate in chicken houses so as to furnish a source of some vitamins, including the little understood animal protein factor.

The importance of coprophagy in animal nutrition experiments has recently been emphasized by Geyer et al. (173), who point out that using raised screen floors in cages is not an adequate preventive measure. Rats and rabbits may frequently be observed eating the fecal pellets as they leave the body. These workers found that when coprophagy was
completely prevented by use of a special cage, growth was sharply curtailed. The growth of controls in screen floor cages was not markedly affected by the diet.

A line of attack which has not been utilized is in the rearing of animals with a bacteria-free intestinal tract. This type of study might result in a positive conclusion concerning the necessity of intestinal microorganisms in the nutrition of intestinal helminths if the effects on the host can be controlled. An exhaustive review of the methods involved has been presented by Glimstedt (177).

Different intestinal helminths might be expected to have varying degrees of dependence on the biochemical activities of intestinal microorganisms. Those forms lacking a digestive tract, the cestodes and acanthocephalans, might obtain significant amounts of synthesized micronutrients. Tissue feeding forms, such as *Nippostrongylus*, and blood-suckers, such as *Ancylostoma*, probably reap little benefit in the form of nutritional factors, but might be affected by bacterial conditioning of the physical properties of the intestinal contents. Those nemas normally feeding on the contents of the intestine, such as *Ascaris*, may be most benefited by bacterial activity. These forms may be able to obtain vitamins from bacterial cells by digestive activity. If we may engage in further mental wandering concerning *Ascaris*, a titillating suggestion comes to mind. There is a possibility that *Ascaris* may have an intra-intestinal bacterial population which supplies the worm with growth factors and/or alters ingested food materials so that these may be more readily utilized by the worm. There is no very strong reason for assuming that the physico-chemical characteristics of the intestinal lumen of *Ascaris* are the same as those of the ex-
ternal environment. We may envisage the possibility that *Ascaris*, and perhaps other nematodes, has a *characteristic* intestinal fauna as do higher animals.

Studies which may contribute toward a further understanding of the activities of intestinal bacteria are being conducted in various laboratories. We know *enough* at this time to conclude that the bacterial ecology of the gut is quite complex and is not well understood.
VI. CERTAIN FEATURES OF THE HOST-PARASITE RELATIONSHIP

In the light of the data which have been presented concerning the nature of the intra-intestinal environment, a reconsideration of certain recent contributions to our knowledge of helminth physiology is in order.

Some phases of the physiology of intestinal helminths have been thoroughly reviewed elsewhere (64, 65, 74, 228, 294, 321, 464) and will not be discussed here. These include the effects on helminths of pH, osmotic pressure, and immunological responses of the host. The following discussion is not intended to represent a comprehensive coverage of recent work, but may serve to indicate the basis for certain general conclusions regarding methodology and to emphasize the need for understanding the host physiology in order to understand the physiology of the parasite in vivo.

A. Effects of Bile Salts

Davey (108) investigated the effects of certain bile salts on sheep nematodes. He reported that Trichostrongylus colubriformis and T. vitrinus, which inhabit the duodenum, showed more resistance to sodium taurocholate and glycocholate than species of Nematodirus, Cooperia, and Oster tagia from the lower small intestine or abomasum. Davey also presented some evidence to show that there might be a differential susceptibility of these nemas to taurocholate or glycocholate.

Some study has been made of the effects of bile salts on
cestodes. DeWaele (116) postulated that *Taenia pisiformis* is able to infect dogs because of the absence of sodium glycocholate in the bile of this host. According to DeWaele, glycocholate is toxic to this cestode and, therefore, the worm will not prosper in hosts whose bile contains this substance. Some recent work in this laboratory indicates that bile from the dog may have a deleterious effect on *Hymenolepis diminuta* (41).

There is some evidence that bile salts may be concerned in causing the evagination of tapeworm scolices in the gut of the definitive host (132). Kent and Macheboeuf (256, 257) have recently reported the interaction of protein fractions from *Moniezia expansa* with some substance which gave a positive Pettenkoffer test. This finding suggests, but does not prove, that bile acids may be involved. As indicated in the foregoing discussion of the bile, there is good evidence that there are quite definite differences in the bile acids of various animals. These differences may be of greater significance in the determination of host specificity than might be thought and would merit further investigation.

### B. The Effect of Milk Sugar in the Host Diet:
#### A Special Case

A number of workers have reported that lactose or milk diets have a marked effect on intestinal nematodes (6, 460, 469) and on cestodes (43, 198). The writer has found no suggested explanation for this effect of milk or milk sugar. Some special effects of lactose on the intestinal environment have been mentioned in foregoing portions of this paper; it is probable that milk sugar exerts its effect indirectly by
alteration of the environment, rather than by a direct effect on intestinal worms. It has been noted that a high lactose diet causes marked alterations of the pH and oxidation-reduction potential of the intestinal contents, as well as changes the intestinal emptying time (152) and has a definite effect on vitamin synthesis by intestinal microorganisms. Alteration of one or all of these factors by high milk sugar diets probably have detrimental effects on intestinal helminths.

C. Vitamin Requirements of Intestinal Helminths

Studies on the vitamin needs of intestinal helminths have all been conducted by experimentally altering the diet of the host or by chemical study of the vitamin content of parasite tissues.

About twenty-five years ago Ackert and his colleagues began investigation on the effect of host dietary vitamin deficiencies on the nematode Ascaridia galli. A major product of these studies was the demonstration that vitamin deficiencies of the host tended to lower resistance to infection with this nematode. This work and related studies on other helminths have been reviewed by Ackert (3) who concluded that "in general the natural resistance is lowered to helminth infections both in the somatic and intestinal phases, when omnivorous hosts are maintained on diets deficient in vitamins A, B (complex), or D."

It might then appear that any deficient diet that lowers the resistance of the host will be beneficial to its helminth parasites. Ackert (3) pointed out that it is not so simple as this. Under some conditions the intestinal environment may be-
come so unfavorable that the lowered resistance of the host does not act in a sufficiently compensatory manner to allow the parasite to prosper. It thus becomes apparent that in any feeding experiment two quite dissimilar effects must be differentiated. One is the change wrought in the resistance of the host and the other is the direct nutritional deficiency of the parasite. It is not always possible to make this differentiation. In general, in helminthic infections in which a readily demonstrable immunity develops, the lowering of resistance apparently outpaces the rate at which parasite nutritional deficiency is produced. Resistance is used here in the sense of immunological resistance, i.e., production of antibodies and anthelminthic resistance, such as the duodenal mucus factor which may differ from antibody production as we understand it at present.

At this time, little can be said regarding the vitamin needs of nematodes. Ackert's experiments (2) indicated that host dietary deficiencies of vitamins A, B-complex, or D had no adverse effect on the growth of *Ascaridia galli* and Ackert concluded that this nematode does not require these vitamins in the first third of its growth period. It would appear that a more exact conclusion would be that this nema is independent of the host diet as a source of these vitamins.

Sadun, et al. (430) have recently reported experiments in which chickens were infected with *Ascaridia* and fed diets deficient in pteroyloglutamic acid. There were more and longer worms in the deficient animals than in the controls. This was interpreted to mean that pteroyloglutamic acid deficiency brings about a "lowered natural resistance" to this parasite. In other experiments Sadun and his coworkers (430) found that on a purified diet, apparently adequate for the host, growth of *Ascaridia* was inhibited. Addition of liver to
the purified diet resulted in some, but not complete restoration of growth. Sadun et al. stated, "These results indicate that there is a substance present in the liver extract necessary in relatively large amounts for the normal growth of the worms. This substance may be vitamin B₁₂ or the animal protein factor."

In the experiments of Sadun et al. it might have been said that the absence from the host diet of some substance, present in liver extract, brings about an increased natural resistance to this parasite. This is so if we assume that the term *host resistance* includes any mechanisms which adversely affect the parasite or which tend to protect the host from injury by the parasite (88). This is not mere quibbling over words. It is simply used to emphasize that understanding of the nutritional requirements can be attained only if we separate the host and parasite physiology for purposes of study.

Can we assume from the experiments described above that *Ascaridia galli* has no pteroyloglutamic acid requirement? The answer is NO. We can only conclude that *A. galli* is not adversely affected by the absence of this vitamin from the host diet.

Some attempts have been made to study the vitamin requirements of cestodes. Hager (198) studied the egg output of *Hymenolepis diminuta* in rats maintained on various diets. Elimination of the B-complex vitamins caused marked inhibition of egg production. Deficiency of thiamine had no significant effect. Chandler (87) studied the effect of various deficient diets on the growth of *H. diminuta*. It was reported that lack of vitamins A, D, and E had no effect on the establishment or growth of the worms. A deficiency of dietary thiamine also had no effect. However, elimination of brew-
er's yeast from the diet caused a marked stunting of the worms in female hosts. Chandler concluded that tapeworms are able to absorb nitrogenous substances, and probably most B vitamins directly from the intestinal mucosa. Addis and Chandler (7, 8) reported further experiments on the effect of host dietary deficiencies on this cestode. It was reported that the worm was independent of vitamins A, D, and E for growth, but that deficiencies of these vitamins reduced the number of worms which became established. The earlier report of lack of effect on the worms of a thiamine-deficient diet was corroborated. In an attempt to determine what, if any, member of the B-complex was concerned in the stunting of the worms observed in animals on a yeastless diet, nine known water-soluble vitamins were added to the diet. The addition of these vitamins did not correct the apparent deficiency. The conclusion was reaffirmed that “part of the vitamins required by the worms are obtained by absorption from the host’s mucous membrane.”

There are some valid objections to the implications in this conclusion. It implies that there is an active transfer of materials directly from the host tissues to the parasite. There are good mechanical reasons which render this highly improbable: (1) The tapeworm is anchored to the host at one end, which allows little or no leverage to be exerted for the intimate contact which would be necessary; (2) The intestine is lined by villi; it is difficult to picture optimum contact of the tip of the moving villus with the worm; (3) The secreting mucosa would tend to keep the worm from actual tissue-to-tissue contact.

It seems more probable that this tapeworm, and perhaps Ascaridia, obtains some of the food materials which it needs by “filching” them from the exocrine-enteric circulation. As
we have noted in the foregoing descriptions of the intestinal secretions, there are sufficient amounts of nitrogenous materials and at least some vitamins to supply the wants of an intestinal helminth. This means that a parasite living in the intestinal lumen has access to materials which may actually be considered a part of the body stores, and may deplete the host just as surely as if direct absorption occurred.

The above hypothesis has been supported by experiments utilizing thiamine which was "labeled" with radioactive sulfur (89). Rats harboring *Hymenolepis diminuta* were placed on a thiamine-free diet and given labeled thiamine parenterally. On autopsy it was found that the thiamine in the tissues of the worm contained the same percentage of radioactivity as the thiamine of the host tissues. This leaves little doubt that adequate thiamine is obtained by this tapeworm from the body stores of the rat in the absence of a host dietary source.

Returning to a point of this discussion, we may ask a question. What is known of the vitamin *requirements* of parasitic worms? Essentially nothing is known. We know that some factor or factors in brewer's yeast, and to a limited extent in liver, is necessary in the host diet for optimum growth and reproduction of *Hymenolepis* in female, immature, or castrated male rats (7, 8, 41, 87). Recent work by Beck (41) indicates that over longer periods of depletion this is true in *all* male as well as female hosts. A similar need of *Ascaridia* for some factor in liver has been demonstrated by Sadun et al. (430). We know that certain vitamins are present in the tissues of various helminths (86, 89, 133, 465). We shall be able to ascertain the requirements only when we can separate the physiology of worm and host. The physiologists will tell us that a vitamin deficiency in a verte-
brate produces profound changes in the physiology. Many of these changes are probably still uninvestigated. Knowledge and control of these unknown factors is necessary before we can conclude that we have produced an avitaminosis in the parasite rather than some deleterious environmental change.

D. EFFECTS OF ENDOCRINE SECRETIONS

Endocrine secretions are known to produce definite changes in the physiology of the gut. However, these relationships have been investigated to a relatively slight extent. A few parasitologists have studied the effects on intestinal worms of the administration of hormones to the host.

There is suggestive evidence that the thyroid may have an effect on intestinal helminths. Todd (492) recently reported that in mildly hyperthyroid hosts, Ascaridia galli attained significantly greater length as compared with worms from normal and mildly hypothyroid birds. Conversely, Heterakis gallinae attained greater length in mildly hypothyroid birds as compared with H. gallinae from normal and mildly hyperthyroid hosts. Percentage development of neither species of worm was significantly different in normal, hypothyroid, or hyperthyroid birds. Larsh (285) reported that if mice were daily given 3 mg. of thyroid extract by mouth for a month prior to infection, a significantly higher percentage of Hymenolepis nana cysticercoids developed than in normal or hypothyroid animals.

Disregarding for the moment the implications of these studies in applied parasitology, we may speculate on the significance of the effects observed. It is noteworthy that in the case of worm species which are apparently benefited by
hyperthyroidism, immunological or anthelmintic host resistance has been demonstrated (3, 5, 428). In Larsh’s experiments with *Hymenolepis nana* there was a lowering of resistance to a tissue-invading phase. Larsh suggested that this might be due to vitamin deficiency induced by hyperthyroidism. On the other hand, in Todd’s experiments with *Ascaridia* (492) there was no evidence of a lowering of resistance to the tissue-invading stage. The experiments suggest that a lowering of production of the mucus anthelmintic factor of Ackert et al. (2, 5) may be involved. This might be associated with the increased rate of intestinal secretion which accompanies hyperthyroidism (151).

Such immunological or anthelmintic resistance has not been demonstrated in the case of *Heterakis*. However, another explanation presents itself in the case of this helminth. It has been established that the thyroid has a stimulating effect on the intestinal absorption of substances which are absorbed by the expenditure of energy through phosphorylation (13). Thus, hypothyroidism slows the rate of absorption of carbohydrates and fats (14). This would conceivably benefit a helminth in the lower digestive tract in one or more ways. It might insure a greater supply of carbohydrate food for the worm. On the other hand, we might expect that the chemosynthetic activity of intestinal microorganisms would be increased because of the presence of additional carbohydrate (see p. 58). Further study of the effect of the thyroid secretion on intestinal physiology seems indicated.

Some data are available on the effect of sex hormones on helminth development. Chandler (87) reported that a diet lacking brewer’s yeast caused a retardation of the growth of the tapeworm *Hymenolepis diminuta* in female rats, but the deficient diet had no deleterious effect on worms from male
rats. This was confirmed by Addis and Chandler (8) who showed that the substance involved was probably not one of the nine water soluble factors known at that time.

Addis (7) reported further studies on a relation between host sex hormones and Hymenolepis. An elaborate series of experiments was performed on the growth of the worm in normal and castrate male and female rats on complete and deficient diets, with and without the administration of hormones, and also in pregnant rats and in immature males and females, with and without the administration of sex hormones. It was again reported that growth of the worms was normal in male rats on a deficient diet but stunted in females on the same diet. In castrate males on complete and deficient diets growth of the worms was retarded. Administration of the testosterone, orally or parenterally, to castrate males on normal or deficient diets produced normal worm growth. In sexually immature male rats on normal or deficient diets the growth of the worms was inhibited. Administration of testosterone to immature males on normal or deficient diets allowed normal worm growth. Injection of progesterone into castrate males also allowed normal worm growth.

In castrate females on a complete diet, worm growth was normal; castrate females on a deficient diet yielded retarded worms. Worm growth was normal in immature females with or without injections of "theelin" and progesterone as long as the host received a complete diet. Injections of "theelin," progesterone, or testosterone, or orally administered testosterone or stilbesterol did not allow normal worm growth in normal female hosts on a deficient diet. In pregnant females on a deficient diet worm growth was normal.

Addis felt that the only definite conclusion that could be
reached was “that the worms are dependent upon testosterone for normal growth in male rats, and that progesterone can be substituted for testosterone.”

Beck (41) showed that a diet lacking brewer’s yeast affected the reproduction of tapeworms in normal adult male rats as well as in other hosts, but found little or no reduction in the size of the worms as was found in female hosts.

Sadun (429) reported that moderate doses of testosterone increased the resistance of young male and female chickens to infection with *Ascaridia galli*. When heavy doses of testosterone were given, the treated animals harbored more worms than the controls. Curves of growth of worms from chickens given testosterone or estradiol were quite different. Estradiol caused an initial retardation and a later increase in the mean growth rate. Conversely, administration of testosterone caused an initial increase and a subsequent retardation of growth rate.

These seem to be the only published studies of the effect of sex hormones on the intestinal phase of parasitic worms. One thing is outstanding in these studies: We have no inkling of the possible ways in which the effects are produced. We know that these hormones have an effect on a variety of physiological functions. Testosterone, for example, changes the metabolic rate of an animal. It affects arginase activity in the intestinal mucosa (263); it increases the synthesis of guanidoacetic acid and creatine (227, 296); it inhibits tone and pendular contraction of the gut (311). Progesterone or pregnancy depresses intestinal motility (148, 500) and may change the rate of glucose absorption (14). A multitude of other physiological features may be involved.
In recent years it has been shown that the rate of emptying of the intestinal tract may have a marked effect on the number of helminths which become initially established or on the position of establishment. This has been shown to be true in the case of *Hymenolepis nana* (285a), *Trichinella spiralis* (287a), and *Ascaridia galli* (4). Larsh (283) has presented evidence that in *H. nana* infections the changes in intestinal emptying time are a factor in altered “resistance” with age and in the lowered “resistance” produced by alcoholism. It was shown that the decreased “resistance” associated with alcoholism was corrected by vitamin administration. This is of interest since B-complex deficiency is usually accompanied by intestinal atonia. Larsh (287) has recently reported a decreased “resistance” to *H. nana* during pregnancy. In this case an altered intestinal emptying time may again be a factor in lowering the “resistance” to initial infection; pregnancy is usually accompanied by intestinal atonia (500).

The intestinal emptying time may be of some importance in the chemotherapy of intestinal parasites. As a case in point, Harwood (210) recently reported that the dose of phenothiazine effective against *Heterakis* in chickens is lower for heavy birds than for light birds. This suggests that intestinal emptying time may be a factor. Artificially increasing the intestinal emptying time along with anthelmintic drug administration might bear investigation as a possible tool in the chemotherapeusis of intestinal helminthic infections.
VII. SOME GENERAL INFERENCES

IT IS believed that enough data have been reviewed to allow the formulation of the following general statements:

(1) The physico-chemical nature of the intestinal environment is extremely complex and is in dynamic relation with the host tissues. Schopfer’s conclusion (445) that physico-chemical factors in the environment are not responsible for host specificity of intestinal helminths is not yet, and may not be, justified. It is likely that Davey’s view (108) is more nearly correct: “Host specificity, or resistance to initial infestation, is primarily an expression of the effect on parasites of the passive environmental conditions provided by the host, and so, too, is their localization in different regions.”

(2) In order to understand the host-parasite relationships of intestinal helminths we must separately investigate the physiology of host and parasite. Following such study, a resynthesis or merging of host and helminth physiology may then reveal entirely new concepts relative to intestinal parasitism.

(3) It follows that it is imperative to develop techniques for cultivating intestinal helminths in vitro, and to make further study of intestinal physiology. It seems apparent that the cultivation of parasitic helminths outside the host presents one of the most difficult and most challenging problems facing parasitologists today.
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