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With 3 Curves and Plates 15, 16, and 17.

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THESE work was conducted by us with the same division of labour as the research on the intestine, its appendages and ferments in the scorpion, published previously. E. Pavlovsky undertook the zoological part of the work—the dissection of live bees, the preparation of the intestine, and the preparation of extracts from its parts. The chemical investigation of the ferments of these extracts was subsequently done by E. Zarin.

ANATOMICAL PART.

The intestine of the bee formed the subject of investigation for many scientists (for literature cf. Zander, Snodgrass), therefore its general anatomical relations may be considered to be sufficiently elucidated.

We shall limit ourselves to the description of the general organization of the intestine and point out some peculiarities in its microscopical structure, whilst the literature on the question will be omitted.

The intestine of the bee consists of the fore-, mid-, and hind-guts (Pl. 15, figs. 1-3). The fore-gut begins with the pharynx, which passes to the oesophagus dilating into the honey-stomach, crop, or ingluvies (Pl. 15, figs. 1-3; Pl. 16, fig. 4, i). The latter passes by means of the cardial valve into the ventriculus (mid-gut or stomach, -v). The hind-gut is divided into the anterior portion—the small intestine (it), and the posterior—the large intestine (-r) with the rectal glands (rg).

The Malpighian vessels (mp) open on the border of the ventriculus and the small intestine.

Fore-gut.

The Fore-gut (pharynx, oesophagus, ingluvies) is lined within by a chitinous cuticle, to the exterior of which lies a layer of non-glandular epithelium (Pl. 16, fig. 5, ep) resting on membrana basilaris. The latter is covered by a network of transversally striated muscle-fibres lying in two layers—circular and longitudinal (Pl. 16, fig. 5, m4, m5).

The valve of the ingluvies is represented by a capitulated
eminence of the bottom of the ingluvies. The capitulum consists of four valves between which there is a cruciate slit (Pl. 16, fig. 5, pc). The valve is provided with three systems of muscular fibres—two longitudinal (Pl. 16, fig. 5, $m_2, m_3$) and one circular (Pl. 16, fig. 5, $m_1$) between them. The former serve to open the valve, the latter to close it. The capitulum of the valve is set on a trunk connecting it with the stomach. From the circumference of the ventricular opening into the intestine hangs an intestiniform cardial valvule preventing the contents of the stomach from returning into the crop. All these data were already established by previous investigators.

Mid-gut (Stomach).

The stomach of the bee consists of a fairly thick cylindrical tube with numerous circular constrictions on it corresponding to which the epithelium of the stomach protrudes into its cavity in the form of folds. The epithelium consists of cylindrical cells which assume the shape of clubs on the ridges of the folds. At the bottom of the depressions between them are situated round groups of cells called cryptae. Exteriorly to the membrana basilaris is disposed the connective tissue in the form of small groups of cells. The muscular membrane of the stomach is formed by two layers of transversally striated muscle-fibres—interior circular, and exterior longitudinal.

(a) The epithelium of the stomach consists of cells with an alveolar protoplasm of basophil character (Pl. 16, fig. 7, $ep$; figs. 9, 11, 13, $ep$). The oval nucleus with sparse chromatin granules, or a dense network of them, lies in the middle of the cell. In its protoplasm are produced oxyphil granules of secretion which are numerous in the superficial portion of the cells. In some sections the cells appear to be set on thin peduncles and to have truncated apices. This picture, as well as the formation of evaginated swellings on the surface of the cells, is in most cases artificial (Pl. 16, fig. 12, $bl$).

The superficial layer of protoplasm of the epithelium is transformed into a fairly broad band vertically striated and bearing the aspect of a brush of cilia (Pl. 16, figs. 9, 11, 12, $wp$).
This hairy layer of protoplasm stains with iron haematoxylin in a grey colour, whereas the protoplasm remains black. The hairy band is covered above by a cuticle (not chitinous); together with the latter it is cast off into the cavity of the stomach in the form of a peritrophic layer (Pl. 16, figs. 6, 7, p; figs. 9, 10, p). This casting off is repeated many times, on account of which in the stomach the membranes are disposed in concentrical layers, sometimes in very great numbers (Pl. 16, fig. 6, p).

The peritrophic membrane presents a structure known for a long time in the articulated animals. With regard to the bee Petersen has demonstrated that the said membrane of this insect contains a proteolytic ferment. The significance of the peritrophic membrane is interpreted in different ways. Some investigators believe it to serve for the defence of the tender stomach epithelium against mechanical injury by vegetable food, especially by the flower pollen in the bee.

Such an interpretation cannot be extended to all Arthropods, since an analogous formation is also present in blood-sucking forms (Culex, Anopheles, according to Schaudinn), the liquid food of which cannot do any harm to the walls of the stomach. Probably those investigators are right who regard the peritrophic membrane as a cuticle formed by the secretion of the stomach epithelium. Originating by transformation of the surface protoplasm of the cells, the membrane itself presents a hard secretion. In the depth of the hairy layer, as in a sponge (Pl. 16, fig. 9, p), is retained the liquid secretion of the stomach, on account of which the same quantity of ferment is capable of acting for a longer period upon the food contained in the cavity of the mid-gut. On account of the relative shortness of the intestine this mode of action of the ferments is of special significance, especially in herbivorous insects, since the food does not pass through the intestine so rapidly, being detained in the folds soaked with the digestive juices of the peritrophic membranes. Thus, in our opinion, they compensate the relatively small length of the intestine in insects.

(b) At the bottom of the folds of the stomach epithelium
are situated groups of smaller cells forming crypts which are weakly developed in the bee.

The cells of the latter are disposed in two or three layers (Pl. 16, fig. 8; fig. 9, k). The deepest row situated on the basal membrane is represented by the smallest cells (in the section three or four of them are visible), covered above and laterally by larger cells bordering directly on the epithelium of the folds of the stomach (Pl. 16, fig. 11, k). In general the protoplasm of the cells of the crypts are more basophil than the stomach epithelium (Pl. 16, fig. 11, k, d). The nuclei of the cryptic cells are large and poor in chromatin. As described by Petersen we did not succeed in observing their karyokinesis. Nasonov (1898), however, observed the process of division of the nuclei in these cells.

The cryptal cells present the sources from which the stomach epithelium is newly formed. Besides, their cells seem to produce a secretion themselves as well. The following facts confirm this supposition. The most superficial cells of the cryptal are not adjacent to each other with their apices, so that there remains an ovoid lumen between them in the shape of a vacuole filled up with a drop of homogeneous secretion staining pink with Giemsa's stain (Pl. 16, fig. 7, k; fig. 11, vc). Besides, there is also observed an accumulation of secretion above the crypta which is revealed by displacement to the sides of the 'hairs' of the superficial band of the stomach epithelium (Pl. 16, fig. 9, k).

In general the secretory processes in the stomach of the bee take the following course:

(1) Separation of the peritrophic membrane (Pl. 16, fig. 6, p; fig. 9, p), (2) production of secretion by the surface of the glandular cells, (3) severance of the superficial portions of the epithelial cells (Pl. 16, fig. 8, d), and (4) separation of a homogeneous secretion by the cryptic cells (Pl. 16, fig. 11, vc).

(c) The epithelium of the stomach lies on a basal membrane clothed exteriorly by a transversely striated muscular membrane, the muscle-fibres of which are very rich in sarcoplasm. The muscle-fibrils are disposed in bundles occupying the greater
part of the surface of the transverse section of the fibre (Pl. 17, fig. 15, \(mf\)) at the point where the sarcoplasm and nuclei are scarce, and half the diameter of the fibre where the sarcoplasm and nuclei are strongly developed. The nuclei always lie in the sarcoplasm nearer to the periphery of the muscle-columns (Pl. 16, fig. 14; Pl. 17, fig. 15, 16, \(sp\)), and not between the latter as in the analogous membrane of the small intestine (Pl. 17, fig. 18).

**Hind-gut.**

The hind-gut is divided into two parts—both in its anatomical and histological structure—the anterior—small intestine (Pl. 15, fig. 3; Pl. 16, fig. 4, \(it\)), and posterior—large intestine (Pl. 16, fig. 4, \(r\)).

**Small Intestine.**

The structure of the small intestine has already been established by previous investigators. We may add to these some details in the microscopical structure of its single-layered cylindrical epithelium. The cells of the latter are covered on their interior surface by a thick chitinous cuticle. The protoplasm of the cells is divided into two portions, the exterior—granular (Pl. 17, fig. 17, \(d\)), and interior—characterized by a rod-line striation (Pl. 17, fig. 17, \(ds\)).

The fairly large rounded nucleus (\(n\)) lies nearer to the base of the cell. Interiorly to it in the layer of granular plasm are found large vacuoles with granules of secretion (\(vs\)). Both the protoplasm and secretion of the epithelium of the small intestine are oxyphil.

The basiliary membrane of the intestine (Pl. 17, fig. 17, \(mb\)) is surrounded by circular muscle-fibres anastomosing with each other. They are thick and their nuclei are disposed along the axis of the fibres surrounded from all sides by bundles of myofibrils (Pl. 17, fig. 18, \(cmf\)).

In general the small intestine of the bee is characterized by the glandular character of its epithelium. The structure of the intestine described may serve as evidence either of its glandular function or of processes of absorption taking place.
in it, or, lastly, of its excretory rôle. We have hitherto only established one fact for certain—the complete absence of ferments in extracts from the small intestine of the bee.

**Large Intestine.**

The large intestine, similarly to the crop of the bee, presents a thin-walled sac which is capable of expanding to enormous dimensions, as seen by comparison of figs. 1 and 3 of Pl. 15. During the whole winter the bees do not evacuate their excrements, but continue taking food, on account of which their large intestine becomes overfilled with faeces and swells into a voluminous bladder.

The scheme of structure of the large intestine is the same as in the crop. In the anterior third of its wall are situated six elongated cylindrical rectal glands (Pl. 17, fig. 19, rg), the microscopical structure of which was in general features correctly described by Snodgrass and Petersen.

We have also succeeded in establishing certain interesting details elucidating the structure of these glands. From the part of the cavity of the rectum each gland is covered by a chitinous cuticle forming on the periphery of the organ a marginal fillet. Within the gland there is an axial cavity (Pl. 17, fig. 21, h) dividing it into two parts—an exterior thin wall (wa) and interior thick one (sn). The latter is formed by tall wedge-shaped cells polyhedral in transverse section.

The exterior wall is formed by two layers of minute polygonal cells (Pl. 17, fig. 21, wa). At the point where both walls join together there lies a syncytial layer of cells containing pigment inclusions (Pl. 17, fig. 21, sn).

The exterior wall of the rectal gland (Pl. 17, fig. 20; fig. 22, wa) is perforated in some places by tracheae (tr) which pass into the cavity of the organ and penetrate with their branches into its inner wall.

To these data, which are to be found in the literature, we may add the following:

The ramifications of the tracheae passing to the thick inner wall of the rectal gland pass along the edge of the poly-
hedral epithelial cells (Pl. 17, figs. 23, 25, tr). The layers of protoplasm of the latter adjacent to the tracheae consist of a substance staining deep black with Heidenhain’s iron haematoxylin (Pl. 17, figs. 22, 25, z).

These bordering layers differ from the alveolar-granular protoplasm of the cells in their dentate aspect; in some individuals they resemble coarse intercellular bridges; in others they are more weakly expressed; their striation, however, is always visible in a greater or less degree.

It is possible to trace the course of the tracheae to four-fifths of the height of the cells. At this level the tracheae which have hitherto pursued a radial course give off lateral branches forming beneath the inner surface of the gland a network rich in anastomoses (Pl. 17, fig. 24, tr). We did not observe anything like the opening of the tracheae directly into the cavity of the intestine in the rectum of the bee, as was described by Vallé in Diptera.

The protoplasm of the large cells is in general granular in some individuals with a fairly distinctly expressed alveolar structure. The protoplasm is oxyphil. To the chitinous cuticle is adjacent a layer of protoplasm staining less and bearing the aspect of vesicles lying close to each other. The nuclei of the cells described are of an irregular round shape. They are disposed either in the middle part, or basally, depending upon the degree to which the protoplasm is filled up with granular inclusions. The nuclei are poor in chromatin.

The variation in the contents of the cells described probably is in connexion with the seasons of the year. In the hibernating bee the large intestine of which had for several months been filled up with faeces, the protoplasm of the large cells of the rectal glands contains numerous globular inclusions and minute granules (Pl. 17, fig. 21, gr). Both are oxyphil, with the exception of some of the larger granules. In some of them are visible roundish portions not stained black with iron haematoxylin. All these formations occupy the middle two-thirds of the transverse section of the cell; whilst in the basal quarter of it lies the displaced nucleus (Pl. 17, fig. 21).
The protoplasm of the rectal glands of bees taken in ordinary condition, although granular, is devoid of the inclusions described above (Pl. 17, fig. 22, d).

In bringing together these facts, we may speak of the absorptive rôle of the rectal glands, which appears to be correct a priori, on account of the long period during which the faeces remain in the rectum in bees hibernating in our latitudes. The microscopical structure of the tall epithelium of the gland points to a possibility of true glandular processes taking place in it. Below we shall discuss the conclusion according to which the rectal glands present the source of seasonal production of catalase, and the point of development of energetic oxidizing processes which is evinced by the intimate connexion between these organs and the tracheae.

Physiological Part.

There are few data in literature regarding the ferments found in the organism of the bee. The first works in this direction were conducted by Erlenmeyer and Planta in 1877.

The authors named dissected 152 worker-bees separating head, thorax, and abdomen, and infused them separately in glycerine. It was found that all the three extracts converted starch to dextrin and sugar, and saccharose to inverted sugar, the extracts from the head and abdomen being much more active than that from the thorax. The extracts from the head and abdomen also contained a ferment dissolving fibrin of the blood, the latter extract being stronger than the former, whilst that from the thorax produced no effect.

The methods applied by Erlenmeyer and Planta for the preparation of extracts is of no use at all, since the exterior division of the body of the bee into head, thorax, and abdomen does not correspond at all to the division of the intestine into its characteristic portions.

In 1912 Petersen, whilst studying a question on the digestion in the bee, also made experiments on the determination of ferments in the digestive organs of the bee. In glycerine extracts from the stomachs of bees the author discovered the
following ferments: diastase, invertase, and a proteolytic ferment dissolving fibrin and splitting peptone. These are essentially all the data to be had in literature on the question discussed.

Methods used in Preparing the Material for Chemical Investigation of the Ferments.

The only fit material is presented by live bees, live for anatomical purposes. They are chloroformed and dissected in physiological solution (0.75 per cent.) of common salt for the preparation of the intestine. The removed intestine is washed in a Petri dish with the physiological solution and separated into the following parts: crop, stomach, small and large intestines.

Each portion is further dissected in order to remove its contents, washed in a fresh portion of the same solution, and placed in a small evaporating glass with a small quantity of desiccated sand and several drops of glycerine. After the portions of the intestine of all the bees have been placed in glasses, they are ground with a glass mortar until a uniformly opaque emulsion is produced. Then to each glass is added the necessary quantity of glycerine or some other liquid, the whole is rapidly mixed and poured out into a jar with a hermetically closing glass stopper.

Extracts were prepared with (a) glycerine, (b) distilled water, (c) a mixture of equal quantities of the liquids named, and (d) physiological solution of common salt. As an antiseptic a few (5–10) drops of toluol were added. The jars were several times shaken thoroughly and left to stand in the darkness at the temperature of the room for different periods.

The density of the extracts varied. We started from strong extracts, ninety bees per 80 c.c. of liquid, i.e. from each portion of the intestine taken from ninety bees an extract was prepared in 30 c.c. By degrees as the experiments progressed it proved to be more practicable to take weaker extracts, until finally we stopped at the proportion of 25 bees per 50 c.c. of liquid.
Altogether thirty analyses were performed, a table relating to the periods of which is adduced below.

### Table I.

<table>
<thead>
<tr>
<th>No. of Experiment</th>
<th>Date of Preparation of Extract</th>
<th>No. of Bees from which Intestines were prepared</th>
<th>Volume of fluid in c.c.</th>
<th>Base on which Extract was prepared</th>
<th>Amount of Total in Drops</th>
</tr>
</thead>
<tbody>
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<td>23 x 1916</td>
<td>90 workers</td>
<td>30</td>
<td>Glycerine</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>7 iv 1917</td>
<td>60</td>
<td>25</td>
<td>Water</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>22 vi 1917</td>
<td>60</td>
<td>25</td>
<td>Glycerine</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>30</td>
<td>12.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>20 vii 1917</td>
<td>50 drones</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>21 ix 1917</td>
<td>40 workers</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>15 xi 1917</td>
<td>30</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>31 m 1918</td>
<td>30</td>
<td>30</td>
<td></td>
<td></td>
</tr>
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<td>30</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>14 iv 1918</td>
<td>15</td>
<td>15</td>
<td>7.5 c.c. glycerine + 7.5 c.c. water</td>
<td>5</td>
</tr>
<tr>
<td>11</td>
<td>15 iv 1918</td>
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<tr>
<td>17</td>
<td>2 viii 1918</td>
<td>15</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>3 vii 1918</td>
<td>15 drones</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td></td>
<td>25 workers</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>6 viii 1918</td>
<td>25</td>
<td>50</td>
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</tr>
<tr>
<td>21</td>
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<td>15</td>
<td>15</td>
<td>7.5 c.c. glycerine + 7.5 c.c. water</td>
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</tr>
<tr>
<td>22</td>
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<td>20 viii 1918</td>
<td>25</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td>15</td>
<td>15</td>
<td>25 c.c. glycerine + 25 c.c. water</td>
<td>10</td>
</tr>
<tr>
<td>26</td>
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<td>15</td>
<td>Water</td>
<td>5</td>
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<td>Glycerine</td>
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<td>Physiol. sol. NaCl</td>
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<td>29</td>
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<td>60</td>
<td>7.5 c.c. glycerine + 7.5 c.c. water</td>
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<tr>
<td>30</td>
<td></td>
<td>25</td>
<td>60</td>
<td>25 c.c. glycerine + 25 c.c. water</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>795 bees</td>
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</tbody>
</table>

As the primary aim of the present work we regarded the qualitative determination of the ferments in the different portions of the ventriculo-intestinal tract of the bee, omitting, meanwhile, the investigation of the ferments of their salivary glands.

In the intestine were established catalase, inulase, lactase, invertase, lipase, pepsin, trypsin, chimosin, and emulsin. Of these ferments we have investigated in fuller detail the catalase and invertase.
Catalase.

As is known, catalase is a ferment widely distributed in the animal and vegetable kingdom. Regarding its presence in the body of the bee no data are known in the literature.

For the determination of catalase we employed a special apparatus constructed by one of us (Zarin).

The process consisted in mixing 2 c.c. of corresponding extracts of ferments with 8 c.c. of water; to the filtered mixture were added 10 c.c. of freshly prepared 1 per cent. solution of hydrogen peroxide; the number of c.c. of oxygen evolved being marked after the expiration of twenty-four hours.

It need not be mentioned that all the analyses were accompanied by control experiments. The results obtained in the investigations are shown in Table II.

TABLE II. Catalase in the Intestine of the Bee.

<table>
<thead>
<tr>
<th>No.</th>
<th>Date of Experiment</th>
<th>Concentration and Composition of Extract</th>
<th>Quantity of Oxygen evolved in c.c.</th>
<th>Crop.</th>
<th>Stomach</th>
<th>Small Intestine</th>
<th>Large Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8 iv 1917</td>
<td>60 bees : 25 c.c. glyc.</td>
<td>0</td>
<td>6-5</td>
<td>0</td>
<td>2-0</td>
<td></td>
</tr>
<tr>
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<td>23 vi 1917</td>
<td>30 bees : 12-5 c.c. glyc.</td>
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<td>1-5</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10 xi 1917</td>
<td>30 bees : 15 c.c. glyc. + 15 c.c. water</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1 iv 1918</td>
<td></td>
<td>0</td>
<td>11-5</td>
<td>0</td>
<td>3-5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>30 bees : 30 c.c. water</td>
<td>0</td>
<td>9-5</td>
<td>0</td>
<td>2-0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>15 iv 1918</td>
<td>15 bees : 7-5 c.c. glyc. + 7-5 c.c. water</td>
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<td>7-3</td>
<td>0</td>
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<td>22 iv 1918</td>
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<td>0</td>
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<td>24 iv 1918</td>
<td></td>
<td>0</td>
<td>7-0</td>
<td>0</td>
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<td></td>
</tr>
<tr>
<td>10</td>
<td>19 vi 1918</td>
<td></td>
<td>0</td>
<td>9-2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>2 vii 1918</td>
<td>25 bees : 25 c.c. glyc. + 25 c.c. water</td>
<td>0</td>
<td>3-0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>4 vii 1918</td>
<td></td>
<td>0</td>
<td>2-0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>15 drones : 7-5 c.c. glyc. + 7-5 c.c. water</td>
<td>0</td>
<td>2-5</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>7 viii 1918</td>
<td>25 bees : 25 c.c. glyc. + 25 c.c. water</td>
<td>0</td>
<td>8-5</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>21 viii 1918</td>
<td></td>
<td>0</td>
<td>4-0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

1 In experiment no. 8 the bees were taken three hours after the hives were removed from the hibernating quarters, when the contents of the rectum were discharged by them.

It is seen from the table that catalase is a specific secretion of the stomach in the bee and drone. This portion of the
intestine produces it continuously during the whole year, whereas in the rectum it was observed in our experiments only in spring.

The latter circumstance is explainable by the fact that the bee remaining in the hive during the six winter months does not discharge its excrements and retains all the faeces till the first spring flight. The discharge of catalase in the rectum depends upon the accumulation of the faeces in it, and evidently serves as a regulation of the different oxidizing processes and destroys the surplus of peroxides in the intercellular metabolism. On the day when the bees issue forth from the hive after hibernation, after three hours of flight during which they become evacuated, the catalase is contained in the large intestine only in a small quantity, and after two days it disappears altogether, as is seen from experiments no. 8 and no. 9 in Table II.

It would be interesting and important from the practical point of view to ascertain the relations presented by catalase in the southern races of bees, which hibernate for a very short period in comparison with our northern bees.

It may be supposed a priori that in the rectum of the southern bees less faeces are accumulated than in northern bees, and that the oxidative processes in the former proceed at a different rate from those in the latter. It is possible that respective investigations would provide an explanation of the failure to acclimatize southern bees in the north. The latter on arriving in the north encounter unusual conditions of a long winter, and have therefore to accumulate excessive masses of faeces in their rectum. It is natural that these bees, unaccustomed to such conditions, are liable to different diseases, amongst which pernicious diarrhoea plays the first rôle.

In order to ascertain whether the secretions from different parts of the intestine stimulate each other when mixed together, catalase was determined in all possible combinations of extracts from parts of the intestines; in each separate case 2 c.c. of the extracts named below were taken:
The experiments prove that in spring, besides the stomach, catalase is produced only by the large intestine (experiments A, C, D). The remaining two portions of the intestine (crop and small intestine) do not produce catalase either separately or combined with others (B); in summer during normal nutrition of the bees this ferment is produced only by the stomach.

In order to study the influence of the time during which the extract from the intestine is infused upon the activity of the catalase, the following experiments were conducted. In one portion the separate parts of the intestine were carefully rubbed together with desiccated sea-sand in the presence of several drops of glycerine; the emulsion produced was diluted with a mixture of equal parts of glycerine and water with the addition of 10 drops of toluol; in another portion quite identical extracts, with the only difference that pieces of intestine were directly placed in a mixture of glycerine and water without being rubbed.

The catalase was determined for periods shown in Table III, in which the results obtained are shown.

An examination of the data represented in Table III reveals the following. Catalase was as usual produced only by the stomach of the bee. The quantity of ferment proved to differ sharply in extracts prepared from rubbed and intact stomachs. In the first extract (no. 17) after half an hour standing there proved to be nearly three times as much ferment as in the second (no. 17 a).

The activity of the ferment in the succeeding days differed in both extracts. In the extract from rubbed stomachs the activity of catalase invariably decreased right to its complete disappearance, which was established by us on the thirtieth day. In the parallel experiment with intact stomachs the
### Table III.

The Influence of the Length of Infusion of the Intestine upon the Activity of Catalase in connexion with the Method of Preparation of the Extract.

<table>
<thead>
<tr>
<th>No.</th>
<th>Date of Experiment.</th>
<th>Concentration and Method of Preparation of Extract.</th>
<th>Part of Intestine.</th>
<th>Length of Infusion of Parts of the Intestine both Rubbed and Intact. c.c. of Oxygen.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$\frac{1}{4}$ hr.</td>
</tr>
<tr>
<td>16</td>
<td>18 vi 1918</td>
<td>15 bees per 30 c.c. of mixture of equal parts of glycerine and water</td>
<td>Rubbed Crop</td>
<td>0</td>
</tr>
<tr>
<td>16a</td>
<td></td>
<td></td>
<td>Intact</td>
<td>0</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td></td>
<td>Rubbed Stomach</td>
<td>9.2</td>
</tr>
<tr>
<td>17a</td>
<td></td>
<td></td>
<td>Intact</td>
<td>3.7</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td></td>
<td>Rubbed Small intest.</td>
<td>0</td>
</tr>
<tr>
<td>18a</td>
<td></td>
<td></td>
<td>Intact</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td></td>
<td></td>
<td>Rubbed Large intest.</td>
<td>0</td>
</tr>
<tr>
<td>19a</td>
<td></td>
<td></td>
<td>Intact</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>2 vii 1918</td>
<td>25 bees per 50 c.c. of mixture of equal parts of glycerine and water</td>
<td>Rubbed Stomach</td>
<td>4.5</td>
</tr>
<tr>
<td>20a</td>
<td></td>
<td></td>
<td>Intact</td>
<td>1.0</td>
</tr>
<tr>
<td>21</td>
<td>4 vii 1918</td>
<td>25 bees per 50 c.c. of mixture of equal parts of glycerine and water</td>
<td>Rubbed</td>
<td>2.0</td>
</tr>
<tr>
<td>22a</td>
<td></td>
<td></td>
<td>Intact</td>
<td>1.0</td>
</tr>
</tbody>
</table>
activity of the same ferment, on the contrary, sharply increased with the duration of infusion of the extract. Thus, already after one day, the active power of catalase increases nearly four times, and on the third day nearly six times; only beginning from the seventh day the activity began to decrease sharply.

Curve 1.

Curve representing the variation of the activity of catalase, according to the length of infusion of the intestine and in connexion with the method of preparation of the extract (series of experiments nos. 17, 20, 21. See Table III).

Such a difference in the activity of catalase in the two parallel experiments described is due, in our opinion, to the mode of preparation of the extracts. When the stomach is finely minced and rubbed, and the cells of its tissues destroyed, a maximum quantity of catalase is freed which gradually becomes inactivated while the extract stands. In extracts from intact pieces of the stomach in which but a small number
of cells were destroyed, when it was cut into parts a smaller quantity of ferment passes into the solution at once, its activity sharply increasing. This increase may be explained in two ways. Either the catalase continues to be produced by the intact cells of the intestine or its amount remains the same, but it only gradually passes from the tissue into the solution according to the time the extract stands.

In the following two parallel experiments (nos. 20, 20a, and nos. 21, 21a) conducted for the control of the data just discussed the picture was somewhat different. In extracts from rubbed stomachs the immediate discharge of catalase in large quantities, as well as its gradual decrease, was corroborated. However, the increase of activity of the ferment in extracts from intact stomachs was exhibited only in experiment no. 21a, in which the activity of the catalase doubled in two days. In experiment no. 20a, however, the activity of the ferment in the extract did not increase from standing, remaining on the same level during five days.

The experiments adduced point to the great variety in the action of catalase which depends on a series of conditions, amongst which the individual character of metabolism in the bee probably occupies the first place.

The solution of these questions should be the subject for special research.

Amylase.

The presence of amylase in the organism of the bee was discovered by Erlenmeyer and Planta and Petersen.

Erlenmeyer and Planta divided the bees into head, thorax, and abdomen, rubbed these parts of their bodies with sand, infused them in glycerine, and established in all three extracts the presence of amylase which converted starch into dextrin and sugar.

Petersen prepared fifty stomachs of bees, rubbed them with sand, infused in 10 c.c. of a mixture of equal parts of glycerine and 1 per cent. of sodium fluoride.

On acting with the extract obtained on starch solution
Petersen arrived at the conclusion that the splitting of starch proceeds to the formation of dextrins. In our experiments to 2 c.c. of corresponding extracts we added 0.1 c.c. of 0.5 per cent. solution of soluble starch, 8 c.c. water, and 2 drops of toluol; the test-tubes with the mixture were then placed for one hour in a water-bath at 45° C. At the expiration of this period the contents of the test-tubes were cooled, and to them iodine solution in potassium iodide was added by drops.

In all the experiments the extracts obtained from the stomachs produced a positive result: after the addition of iodine it always assumed a light-yellow coloration, whereas the extracts from the remaining three portions of the intestine, namely crop, mid-, and hind-guts, contained no amylase and assumed a blue colour after addition of iodine.

Thus our experiments proved that amylase is present only in the stomach of the bee, whereas the remaining portions of the intestine do not produce this ferment. In this case the splitting of starch proceeds not only to the formation of dextrins, as Petersen's experiments have shown, but to the formation of maltose and dextrose.

Owing to the presence of amylase in the digestive stomach the bee can digest starchy food.

The fact that Erlenmeyer and Planta discovered amylase in all the three extracts (from the head, thorax, and abdomen) is explainable on the basis of our findings in the following manner: the amylase in the extract from the abdomen doubtless is derived from the stomach of the bee; the same ferment in extracts from the head and thorax are probably produced by the salivary glands, since the fore-gut (oesophagus) passing through the named part of the body does not produce this ferment.

Inulase.

As is known, inulase converts the polysaccharid-inulin into levulose. Regarding the presence of this ferment in the intestine of the bee there are no data in the literature. The results obtained in our experiments allow us to conclude that the intestine of the bee does not produce inulase.
Lactase.

Lactase is a ferment splitting the disaccharid—milk sugar—into monosaccharids—glucose and galactose. There are no data in literature concerning the production of lactase in the intestine of the bee. In our experiments the presence of lactase was not established.

Invertase.

The presence of invertase in the organism of the bee was first discovered by Erlenmeyer and Planta in glycerine extracts from the heads, thoraces, and abdomens of bees. The extracts from the heads and abdomens prove to be more active than those from the thoraces.

Axenfeld divided the intestine of the bee into three parts—crop, stomach, and hind-gut. On acting with the named portion of the intestine on the solution of saccharase, the greatest activity was exhibited by the stomach, whereas the crop and hind-gut inverted sugar very weakly. The author named supposes that invertase is produced only by the stomach, but a small quantity of it is transferred mechanically to the hind-gut.

In our experiments we added to 5 c.c. of 10 per cent. solution of cane-sugar the tested extracts from the intestine of the bee and drone in quantities shown in the table, the liquid being placed—after addition of 1 c.c. of toluol as a conserving medium—in the thermostat at 36-40° C. for twenty-four to forty-eight hours; then after cooling the liquid to the temperature of the room and adding 1 drop of ammonia, in order to avoid birotation, the rotation of the plane of polarization was determined in a tube of 200 mm.

The results obtained are adduced in Table IV.

On examining the data adduced in the table it is seen that in regard to invertase the first two experiments differ essentially from the remaining, notwithstanding the similar methods employed in the analysis. Whereas in all the experiments, except the first two, the extracts from the stomach show an essential decrease of the right rotation of the polarization plane,
### Table IV.

**Invertase in the Ventriculo-Intestinal Tract of the Worker-bee and Drone.**

<table>
<thead>
<tr>
<th>No. of Experiment.</th>
<th>Date of Experiment</th>
<th>Concentration and Composition of Extracts.</th>
<th>Concentration of Sugar Solution (c.c.)</th>
<th>Quantity of Ferment Extract (c.c.)</th>
<th>Time of Action of Ferment (hrs.)</th>
<th>Degrees of Rotation of the Plane of Polarization</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24 x 1916</td>
<td>90 bees per 30 c.c. of glycerine</td>
<td>100</td>
<td>2</td>
<td>48</td>
<td>+13-25 + 13-22 + 13-23 + 13-24 —</td>
</tr>
<tr>
<td>2</td>
<td>8 IV 1917</td>
<td>60 bees per 25 c.c. of glycerine</td>
<td>5</td>
<td>2</td>
<td>48</td>
<td>+6-13 + 6-12 + 6-10 + 6-13 + 6-14 —</td>
</tr>
<tr>
<td>3</td>
<td>23 vi 1917</td>
<td>30 bees per 12-5 c.c. of glycerine</td>
<td>5</td>
<td>5</td>
<td>24</td>
<td>+6-23 + 6-23 + 2-88 + 6-26 + 6-23 —</td>
</tr>
<tr>
<td>4</td>
<td>23 vi 1917</td>
<td>60 bees per 25 c.c. of water</td>
<td>5</td>
<td>5</td>
<td>24</td>
<td>+6-28 + 6-24 + 1-30 + 6-27 + 6-28 —</td>
</tr>
<tr>
<td>5</td>
<td>21 vii 1917</td>
<td>50 drones per 25 c.c. of glycerine</td>
<td>5</td>
<td>5</td>
<td>24</td>
<td>+6-08 + 6-08 + 5-78 + 6-06 + 6-06 + 6-06 —</td>
</tr>
<tr>
<td>6</td>
<td>22 vii 1917</td>
<td>40 bees per 25 c.c. of water</td>
<td>5</td>
<td>10</td>
<td>48</td>
<td>+11-45 — + 8-90 — — — + 11-28</td>
</tr>
<tr>
<td>7</td>
<td>16 x 1917</td>
<td>30 bees per 15 c.c. of glycerine + 15 c.c. of water</td>
<td>5</td>
<td>15</td>
<td>48</td>
<td>+5-65 + 5-65 + 7-5 + 5-90 + 5-90 —</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>1 iv 1918</td>
<td>30 bees per 30 c.c. of water</td>
<td>5</td>
<td>50</td>
<td>48</td>
<td>+6-12 — + 2-30 — — — + 5-15 —</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>15 iv 1918</td>
<td>15 bees per 7-5 c.c. of glycerine + 7-5 c.c. of water</td>
<td>5</td>
<td>50</td>
<td>48</td>
<td>+6-00 + 5-89 + 5-84 + 5-84 + 5-84 —</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>—</td>
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<tr>
<td>11</td>
<td>16 iv 1918</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>—</td>
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<tr>
<td>12</td>
<td>22 iv 1918</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>13</td>
<td>24 iv 1918</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>—</td>
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<tr>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>15</td>
<td>18 vi 1918</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>—</td>
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<tr>
<td>17</td>
<td></td>
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<td></td>
<td>—</td>
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<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>19</td>
<td>2 vii 1918</td>
<td>25 bees per 25 c.c. of glycerine + 25 c.c. of water</td>
<td>5</td>
<td>50</td>
<td>48</td>
<td>+5-90 — + 5-90 — + 2-18 — — — + 5-62</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>22</td>
<td>4 vii 1918</td>
<td>15 drones per 7-5 c.c. of glycerine + 7-5 c.c. of water</td>
<td>5</td>
<td>50</td>
<td>48</td>
<td>+6-08 + 6-08 + 5-17 + 5-17 + 5-17 —</td>
</tr>
<tr>
<td>23</td>
<td>7 viii 1918</td>
<td>15 bees per 15 c.c. of water</td>
<td>5</td>
<td>50</td>
<td>48</td>
<td>+6-08 + 6-08 + 5-17 + 5-17 + 5-17 —</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>—</td>
</tr>
</tbody>
</table>
in experiments no. 1 and no. 2 the difference between the control and experimental solutions in this respect is so insignificant (0.07° and 0.08°) that it may be ascribed to error in analysis, and it is impossible to draw any definite conclusions from it. However, the great activity of invertase in the remaining experiments points to the absence of this ferment in the extracts tested in the experiments discussed. It is characteristic that in regard to the remaining ferments (amylase, inulase, lactase, lipase, pepsin, trypsin, and chimosin) there is no essential difference between these two and the remaining experiments.

As is seen from the table the two experiments named were conducted late in autumn and early in spring when the bees were in the stage of winter rest and fed on honey which, as is known, consists chiefly of inverted sugar and contains no saccharose at all, or contains it in insignificant quantities. The significance of invertase both in the animal and plant kingdoms lies in its capacity of converting saccharose into inverted sugar directly assimilated by the protoplasm.

Since the ferment is produced by the cells chiefly when the organism requires it, the absence of invertase in the first two experiments are provisionally explained by the fact that the bees feeding in winter on inverted sugar are not in need of it, and do not therefore produce this ferment.

Cases in which the same organism is capable, according to conditions, of different ferment-productive activity are not rare; sometimes the presence of a definite substance specific to the given ferment is quite sufficient to activate it.

Thus, according to Oppenheimer, some mucorines produce no ferments when cultivated in media containing substances assimilated by them directly. However, on addition of proteins to the medium the same mucorine produces proteolytic ferments on addition of starch-amylase, &c.

The investigations of Brown and Moris have shown that the germ of malt does not produce amylase if the grains are cultivated in media containing sugars capable of assimilation.

Therefore, it is possible that the organism of the bee is also
capable of not producing invertase in definite conditions. At any rate, as is seen from the table, the tested extracts of the same concentration manifest different activity.

If circumstances allow we shall dedicate special experiments to the solution of the question discussed.

A further examination of the data adduced in the table shows that only extracts from the stomach possess considerable power of preventing the rotation of the polarization plane in the sugar solution, whereas those from the crop, small and large intestines, are either altogether inactive or act very weakly. Evidently these latter portions of the intestine do not produce invertase, the latter penetrating there from the stomach together with the food.

In experiments nos. 6, 7, 8, 19, and 22, invertase was determined in extracts prepared immediately from the crop, small and large intestines, the alteration in the rotation of the plane of polarization being insignificant.

Thus we have arrived at conclusions coinciding with Axenfeld’s opinion regarding the place in which invertase is produced, i.e. that it is produced in the stomach of the bee.

The data of Erlenmeyer and Planta, according to which greater activity was manifested by extracts from the head and abdomen of the bees than from their thorax, may be explained as follows.

The origin of invertase from the abdomen should be referred to the stomach of the bee. The greater activity of the extract from the head of the bee, as compared with that from the thorax, is explained by the fact that the salivary glands lying in the head produce invertase, whilst those lying in the thorax do not produce this ferment. Such an explanation is, of course, only probable, and must be verified by special investigations.

After having ascertained the general relations exhibited by invertase in the ventriculo-intestinal tract of the bee, we have also conducted several preliminary experiments with the view of a special study of the nature of this ferment.

Thus it was interesting to determine the influence of the method of preparation of the ferment on the activity of inver-
tase. Similarly to the analogous experiments with catalase (see Table III) we prepared extracts from finely rubbed stomachs, as well as from separate pieces of it.

The results obtained from investigation of these extracts are adduced in Table V.

The table shows that the rubbing of the tissues of the intestine does not manifest any visible influence on the activity of invertase, contrary to catalase. The slight difference between the rotation of the polarization planes in extracts from rubbed and intact stomachs is, probably, due to the individuality of the bees.

Further, we were interested in the influence of the quantity of extract on the course of inversion of sugar. For the elucidation of this question we have conducted a series of experiments in which to equal quantities of 5 per cent. solution of cane-sugar were added different amounts of the same extract from stomachs of bees. After standing a day at +36-8° C. the rotation of the polarization plane was determined in the mixture. The results obtained in these experiments are adduced in Table VI.

On comparing the results of the experiments in Table VI it is seen that by degrees as the amount of extracts increases the quantity of inverted sugar in the solutions tested also increases. However, there is no strict proportionality between these increases. In general, as all the analyses have shown, the ferment manifests a greater activity in smaller quantities, whereas with the increase of quantity its activity decreases. For instance, in experiments nos. 33 and 33 a 1 c.c. of extract evoked a diminution of the angle of rotation on 1-68° and 5 c.c. of the same extract only on 3-19°. That the rate of inversion is not strictly proportional to the amount of invertase is also corroborated by Kikkan,¹ who worked on invertase obtained by him from yeast.

A very interesting picture is produced by calculating the number of stomachs corresponding to the respective amount

¹ D. A. Kikkan, 'On the question regarding the process of inversion under the influence of invertin'. Dissertation for the degree of Mag. Pharm. Uriev., 1903, p. 53.
### Table V.

**The Influence of the Degree to which the Stomachs of the Bees were Triturated on the Activity of Invertase.**

<table>
<thead>
<tr>
<th>No.</th>
<th>Date of Experiment</th>
<th>Method of Preparation of Extract</th>
<th>Concentration of Sugar Solution in per cent.</th>
<th>Quantity of Sugar Solution in c.c.</th>
<th>Quantity of Ferment Extracts in c.c.</th>
<th>Time of Action of Ferment at 36-8° C.</th>
<th>Degrees of Rotation of Polarization Plane.</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>16 iv 1918</td>
<td>15 bees per 7-5 c.c. of glycerine+ 7-5 c.c. of water</td>
<td>5</td>
<td>50</td>
<td>5</td>
<td>24</td>
<td>+6-00</td>
</tr>
<tr>
<td>27</td>
<td>18 iv 1918</td>
<td>,</td>
<td>5</td>
<td>50</td>
<td>5</td>
<td>24</td>
<td>+5-87</td>
</tr>
<tr>
<td>28</td>
<td>,</td>
<td>,</td>
<td>5</td>
<td>50</td>
<td>1</td>
<td>24</td>
<td>+5-38</td>
</tr>
<tr>
<td>29</td>
<td>2 vii 1918</td>
<td>25 bees per 25 c.c. of glycerine+ 25 c.c. of water</td>
<td>5</td>
<td>50</td>
<td>6</td>
<td>24</td>
<td>+5-90</td>
</tr>
<tr>
<td>30</td>
<td>,</td>
<td>,</td>
<td>5</td>
<td>50</td>
<td>4</td>
<td>24</td>
<td>+6-08</td>
</tr>
<tr>
<td>31</td>
<td>,</td>
<td>,</td>
<td>5</td>
<td>50</td>
<td>2</td>
<td>24</td>
<td>+6-35</td>
</tr>
</tbody>
</table>

Mixture of Sugar Solution with Extract from:

- Control
- Rubbed Stomachs
- Injected Stomachs

Difference in Degrees of Rotation from + relative to - Rubbed.
### Table VI.

**The Influence of the Amount of Extracts on the Degree of Inversion.**

<table>
<thead>
<tr>
<th>No.</th>
<th>Time of Experiment</th>
<th>Concentration and Method of Preparation of Extract</th>
<th>Concentration of Sugar Solution in c.c.</th>
<th>Quantity of Sugar Solution in c.c.</th>
<th>Quantity of Extract in c.c.</th>
<th>Time of Action of Particles of Extract in Hours</th>
<th>Degrees of Rotation of Polarization Plane</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>15 iv 1918</td>
<td>15 bees per 7-5 c.c. of glycerine + 15 c.c. of water</td>
<td>5</td>
<td>100</td>
<td>5</td>
<td>24</td>
<td>+6-13</td>
</tr>
<tr>
<td>32a</td>
<td></td>
<td></td>
<td>5</td>
<td>100</td>
<td>5</td>
<td>24</td>
<td>+6-32</td>
</tr>
<tr>
<td>33</td>
<td>18 vi 1918</td>
<td></td>
<td>5</td>
<td>50</td>
<td>5</td>
<td>24</td>
<td>+5-87</td>
</tr>
<tr>
<td>33a</td>
<td></td>
<td></td>
<td>5</td>
<td>50</td>
<td>1</td>
<td>24</td>
<td>+6-38</td>
</tr>
<tr>
<td>34</td>
<td>2 vni 1918</td>
<td>25 bees per 25 c.c. of glycerine + 25 c.c. of water</td>
<td>5</td>
<td>50</td>
<td>2</td>
<td>24</td>
<td>+6-36</td>
</tr>
<tr>
<td>34a</td>
<td></td>
<td></td>
<td>5</td>
<td>50</td>
<td>4</td>
<td>24</td>
<td>+6-36</td>
</tr>
<tr>
<td>34b</td>
<td></td>
<td></td>
<td>5</td>
<td>50</td>
<td>6</td>
<td>24</td>
<td>+6-08</td>
</tr>
<tr>
<td>35</td>
<td>7 viii 1918</td>
<td></td>
<td>5</td>
<td>50</td>
<td>0.1</td>
<td>24</td>
<td>+6-30</td>
</tr>
<tr>
<td>35a</td>
<td></td>
<td></td>
<td>5</td>
<td>50</td>
<td>0.3</td>
<td>24</td>
<td>+6-30</td>
</tr>
<tr>
<td>35b</td>
<td></td>
<td></td>
<td>5</td>
<td>50</td>
<td>0.5</td>
<td>24</td>
<td>+6-30</td>
</tr>
<tr>
<td>35c</td>
<td></td>
<td></td>
<td>5</td>
<td>50</td>
<td>0.8</td>
<td>24</td>
<td>+6-30</td>
</tr>
<tr>
<td>35d</td>
<td></td>
<td></td>
<td>5</td>
<td>50</td>
<td>1</td>
<td>24</td>
<td>+6-30</td>
</tr>
<tr>
<td>35e</td>
<td></td>
<td></td>
<td>5</td>
<td>50</td>
<td>2</td>
<td>24</td>
<td>+6-30</td>
</tr>
<tr>
<td>35f</td>
<td></td>
<td></td>
<td>5</td>
<td>50</td>
<td>5</td>
<td>24</td>
<td>+6-30</td>
</tr>
<tr>
<td>35g</td>
<td></td>
<td></td>
<td>5</td>
<td>50</td>
<td>10</td>
<td>24</td>
<td>+6-30</td>
</tr>
<tr>
<td>35h</td>
<td></td>
<td></td>
<td>5</td>
<td>50</td>
<td>25</td>
<td>24</td>
<td>+6-30</td>
</tr>
<tr>
<td>36</td>
<td>21 viii 1918</td>
<td></td>
<td>5</td>
<td>50</td>
<td>0.1</td>
<td>24</td>
<td>+6-30</td>
</tr>
<tr>
<td>36a</td>
<td></td>
<td></td>
<td>5</td>
<td>50</td>
<td>0.3</td>
<td>24</td>
<td>+6-30</td>
</tr>
<tr>
<td>36b</td>
<td></td>
<td></td>
<td>5</td>
<td>50</td>
<td>0.5</td>
<td>24</td>
<td>+6-30</td>
</tr>
<tr>
<td>36c</td>
<td></td>
<td></td>
<td>5</td>
<td>50</td>
<td>0.8</td>
<td>24</td>
<td>+6-30</td>
</tr>
<tr>
<td>36d</td>
<td></td>
<td></td>
<td>5</td>
<td>50</td>
<td>1</td>
<td>24</td>
<td>+6-30</td>
</tr>
<tr>
<td>36e</td>
<td></td>
<td></td>
<td>5</td>
<td>50</td>
<td>2</td>
<td>24</td>
<td>+6-30</td>
</tr>
<tr>
<td>36f</td>
<td></td>
<td></td>
<td>5</td>
<td>50</td>
<td>5</td>
<td>24</td>
<td>+6-30</td>
</tr>
<tr>
<td>36g</td>
<td></td>
<td></td>
<td>5</td>
<td>50</td>
<td>10</td>
<td>24</td>
<td>+6-30</td>
</tr>
<tr>
<td>36h</td>
<td></td>
<td></td>
<td>5</td>
<td>50</td>
<td>25</td>
<td>24</td>
<td>+6-30</td>
</tr>
</tbody>
</table>
of extract. For instance, in experiment no. 35, 0.1 c.c. of extract corresponds to one-twentieth part of the stomach of one bee, and the quantity of ferment contained in this small particle already evokes an alteration of the polarization plane to curve 2.

![Curve 2](image)

Curve representing the influence of the quantity of invertase upon the degree of inversion, according to the data obtained from the series of experiments nos. 35 and 36 (see Table VI).

0.11°. This small experiment clearly exhibits the power of activity of the invertase of the stomach of the bee.

In the more perfect experiments, nos. 35, 36, it was established that extracts added to sugar syrup in large quantities evoke not an increase of inversion, but, on the contrary, its decrease (nos. 35 f, 35 g, and 36 f, 36 g).

This apparently uncommon phenomenon may be due either
to the quantity of glycerine or the concentration of the sugar solution, as we added to 50 c.c. of the latter an amount of extracts varying between 0·1 and 25 c.c. In order to ascertain the real cause of this phenomenon, we conducted special experiments on the following plan. Extracts were prepared simultaneously from the stomachs of bees of equal concentration in water and in a mixture of equal parts of glycerine and water. To 25 c.c. of 10 per cent. sugar solution was added a certain amount of extract (see Table VII), and the mixture resulting made to reach 50 c.c. by addition of a corresponding amount of water or its mixture with glycerine. As a result, in all the tests analysed, the concentration of cane-sugar reached the same level, differing from each other only in the quantitative content of ferment. Thus we have conducted two parallel series of experiments in water and in a mixture of water and glycerine; the tests of one series of experiments differed from the other only in the presence of glycerine in it. The results obtained are given in Table VII.

On comparing the results of analyses adduced in Table VII we can state without doubt that in extracts of glycerine with water inversion increases only to 10 per cent. of the content of glycerine estimated in relation to the total volume of the liquid analysed. On further addition of glycerine the activity of invertase falls perceptibly. In parallel experiments with pure water extracts the degree of inversion rises according to the increase in the quantity of extract, that is to say, of invertase.

Thus, in certain quantities, glycerine has a repressive influence on the invertase as represented in Curve 3.

In order to determine the influence of the solvent upon the activity of invertase extracts from the stomachs of fifteen bees per 15 c.c. of water, 15 c.c. of glycerine, and, lastly, per 15 c.c. of physiological solution of common salt, were prepared in similar conditions; to each extract were added 5 drops of toluol for conserving purposes. On the following day to 50 c.c. of 5 per cent. solution of saccharose were added 5 c.c. of the extracts named and 10 drops of toluol; after twenty-
<table>
<thead>
<tr>
<th>No.</th>
<th>Method of Preparation of Extract</th>
<th>Concentration of Sugar Solution in per cent.</th>
<th>Quantity of Sugar Solution in c.c.</th>
<th>Quantity of Ferment Extract in c.c.</th>
<th>Number of Stomach of Bee per respective amount of Extract.</th>
<th>Volume of Mixture of both.</th>
<th>24 hours</th>
<th>48 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>25 bees per 50 c.c. of mixture of equal parts of glycerine and water</td>
<td>10</td>
<td>25</td>
<td>1</td>
<td>0.5</td>
<td>50</td>
<td>+6.56</td>
<td>+6.29</td>
</tr>
<tr>
<td>37a</td>
<td>&quot;</td>
<td>10</td>
<td>25</td>
<td>2</td>
<td>1</td>
<td>50</td>
<td>+6.56</td>
<td>+6.29</td>
</tr>
<tr>
<td>37b</td>
<td>&quot;</td>
<td>10</td>
<td>25</td>
<td>5</td>
<td>2.5</td>
<td>50</td>
<td>+6.56</td>
<td>+6.29</td>
</tr>
<tr>
<td>37c</td>
<td>&quot;</td>
<td>10</td>
<td>25</td>
<td>10</td>
<td>5</td>
<td>50</td>
<td>+6.56</td>
<td>+6.29</td>
</tr>
<tr>
<td>37d</td>
<td>&quot;</td>
<td>10</td>
<td>25</td>
<td>25</td>
<td>12.5</td>
<td>50</td>
<td>+6.56</td>
<td>+6.29</td>
</tr>
<tr>
<td>38</td>
<td>25 bees per 50 c.c. of water</td>
<td>10</td>
<td>25</td>
<td>1</td>
<td>0.5</td>
<td>50</td>
<td>+6.54</td>
<td>+6.29</td>
</tr>
<tr>
<td>38a</td>
<td>&quot;</td>
<td>10</td>
<td>25</td>
<td>2</td>
<td>1</td>
<td>50</td>
<td>+6.54</td>
<td>+6.29</td>
</tr>
<tr>
<td>38b</td>
<td>&quot;</td>
<td>10</td>
<td>25</td>
<td>5</td>
<td>2.5</td>
<td>50</td>
<td>+6.54</td>
<td>+6.29</td>
</tr>
<tr>
<td>38c</td>
<td>&quot;</td>
<td>10</td>
<td>25</td>
<td>10</td>
<td>5</td>
<td>50</td>
<td>+6.54</td>
<td>+6.29</td>
</tr>
<tr>
<td>38d</td>
<td>&quot;</td>
<td>10</td>
<td>25</td>
<td>25</td>
<td>12.5</td>
<td>50</td>
<td>+6.54</td>
<td>+6.29</td>
</tr>
</tbody>
</table>

**Table VII.**

**The Influence of Glycerine on the Activity of Invertase.**
four and forty-eight hours standing at 36–40° C. the rotation of the polarization plane was determined in the mixtures.

The results obtained are represented in Table VIII.

Curve 3.

Curve demonstrating the depressive effect of glycerine upon the activity of invertase, based on the data of the series of experiments nos. 37 and 38 (see Table VII).

It is seen from Table VIII that invertase is extracted both by water and a mixture of water and glycerine, by glycerine alone, as well as by the physiological solution of common salt.

Evidently the most active extracts are produced by water, less by glycerine. Water, however, can be used effectively
## Table VIII. The Influence of the Solvent upon the Activity of Invertase.

<table>
<thead>
<tr>
<th>No.</th>
<th>Date of Experiment</th>
<th>Method of Preparation of Extracts</th>
<th>Concentration of Sugar Solution in per cent.</th>
<th>Quantity of Ferment Extracts in c.c.</th>
<th>Degrees of Rotation of Polarization Plane at the Expiration of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24 hours.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>48 hours.</td>
</tr>
<tr>
<td>39</td>
<td>7 VIII 1918</td>
<td>15 bees per 15 c.c. of water</td>
<td>5</td>
<td>5</td>
<td>+ 5.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ 2.08</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td>15 bees per 7.5 c.c. of glycerine+7.5 c.c. of water</td>
<td>5</td>
<td>5</td>
<td>+ 5.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ 2.40</td>
</tr>
<tr>
<td>41</td>
<td></td>
<td>15 bees per 15 c.c. of glycerine</td>
<td>5</td>
<td>5</td>
<td>+ 5.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ 3.01</td>
</tr>
<tr>
<td>42</td>
<td>21 VIII 1918</td>
<td>15 bees per 15 c.c. of water</td>
<td>5</td>
<td>5</td>
<td>+ 5.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ 0.53</td>
</tr>
<tr>
<td>43</td>
<td></td>
<td>15 bees per 7.5 c.c. of glycerine+7.5 c.c. of water</td>
<td>5</td>
<td>5</td>
<td>+ 5.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ 2.40</td>
</tr>
<tr>
<td>44</td>
<td></td>
<td>15 bees per 15 c.c. of glycerine</td>
<td>5</td>
<td>5</td>
<td>+ 6.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ 3.25</td>
</tr>
<tr>
<td>45</td>
<td></td>
<td>15 bees per 15 c.c. of physiol. Solut. NaCl</td>
<td>5</td>
<td>5</td>
<td>+ 5.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ 0.25</td>
</tr>
</tbody>
</table>

## Table IX. The Activity of Invertase in Conserved Solutions.

<table>
<thead>
<tr>
<th>No.</th>
<th>Date of Experiment</th>
<th>Method of Preparation of Extracts</th>
<th>Date of Analysis</th>
<th>Concentration of Sugar Solution in per cent.</th>
<th>Quantity of Ferment Extracts in c.c.</th>
<th>Degrees of Rotation of the Plane of Polarization</th>
</tr>
</thead>
<tbody>
<tr>
<td>46</td>
<td>15 XI 1917</td>
<td>30 bees per 30 c.c. of a mixture of glycerine and water (55) and of toluol</td>
<td>16 XI 1917, after day</td>
<td>5</td>
<td>50</td>
<td>+ 5.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+ 2.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.53</td>
</tr>
<tr>
<td>46a</td>
<td></td>
<td></td>
<td>10 IV 1918,</td>
<td>5</td>
<td>50</td>
<td>+ 5.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 months</td>
<td></td>
<td></td>
<td>+ 2.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.50</td>
</tr>
<tr>
<td>46b</td>
<td></td>
<td></td>
<td>15 XI 1918,</td>
<td>5</td>
<td>50</td>
<td>+ 5.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11 months</td>
<td></td>
<td></td>
<td>+ 2.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.44</td>
</tr>
</tbody>
</table>
only in cases which do not require long conservation of the material analysed, since the extracts rapidly decompose. A very suitable solvent for invertase, as regards resistance, is presented by a mixture of glycerine and water. Our experiments have shown that extracts prepared in this mixture retain their original activity at least during eleven months (Table IX). Glycerine, on the other hand, suffers from another defect: it acts depressively on the course of inversion, as is clearly visible from the series of experiments nos. 35, 36, 37 of Tables VI and VII and Curves 2 and 3. As in the preceding experiments invertase manifests its activity in different solvents also only during the first days.

In order to determine the durability of invertase after prolonged conservation of its solutions the following experiment was conducted.

On November 16, 1917, an extract from the stomachs of thirty bees per 30 c.c. of a mixture of glycerine and water, with the addition of 10 drops of toluol, was prepared. This extract, preserved in a dark place at the temperature of the room, was analysed in the usual way on November 17, 1917, April 10, 1918, and October 15, 1918. The results obtained from the analysis are represented in Table IX.

The series of analyses given in Table IX shows that the activity of invertase in solutions prepared in a mixture of glycerine and water does not alter after being preserved for at least eleventh months, in which respect invertase differs markedly from catalase.

**Lipase.**

As is known, lipase belongs to ferments splitting fats into fatty acids and glycerine.

The source of fatty food for the bee is presented by propolis which contains, according to the analysis of one of us (Zarin), about 6 per cent. of fat.

The question whether the fatty substances of propolis are assimilated by the organism of the bee cannot be regarded as
settled, since there are hitherto no data in literature referring to lipase in the bee.

Petersen, on failing to discover fat in the epithelial cells of the digestive stomach of the bee with the help of osmic acid and the stain-sudan III, writes: 'Als sicher darf ich wohl hinstellen, dass das meiste Fett, auch der normalen Nahrung, den Darm passiert, ohne gespalten oder resorbiert zu werden.'

For the detection of lipase we employed 1 per cent. solution of monobutyric and the emulsion of Provence oil.

In all the experiments conducted the same results were obtained in general. An increase in acidity both of the monobutyric solution and the Provence oil emulsion was observed only in those test-tubes that contained extracts from the stomachs; this increase of acidity varied in the experiments conducted between 0.3–0.6 c.c. \( \frac{N}{10} \) NaOH, the ferment acting more intensely on the monobutyric than on Provence oil. The extracts from the remaining portions of the intestine produced no distinct influence upon the acidity of the medium.

Thus, our experiments show that the stomach of the bee and drone produces, together with other ferments, lipase as well; the bee is therefore capable of assimilating fatty substances.

**Pepsin.**

For the determination of pepsin we employed:

1. Sterile 1 per cent. gelatine acidulated with hydrochloric acid to a distinctly acid reaction, and poured 2 c.c. of this into thin test-tubes. To this quantity of gelatine were added 2 c.c. of extracts of ferments tested and 3 drops of toluol; the mixture was placed in the thermostat at 35–8° C., and every twenty-four hours the degree of its coagulation was observed on being cooled.

2. The fibrin from blood. For our purposes we placed a piece of fibrin into a mixture consisting of 8 c.c. of sterile water acidulated with hydrochloric acid to a distinctly acid reaction, 2 c.c. of extracts of ferments tested and 3 drops of toluol; the test-tube being placed in the thermostat at 35–8° C.
(3) 1 per cent. solution of casein containing in 1 litre 16 c.c. of concentrated hydrochloric acid of specific gravity 1.124.

Test-tubes containing 10 c.c. of this solution and 2 c.c. of extracts of the ferments tested were placed for one hour in the water-bath at a temperature of 38–40° C., after which to their contents a concentrated solution of sodium citrate was carefully added by drops.

The methods described produced in all cases similar results, the presence of pepsin being established in the stomachs of the bee and drone, whilst the other portions of the intestine were devoid of it.

(1) Gelatine with extracts from the stomachs of the bee and drone lost the coagulating property after one to three days, whereas extracts from the remaining portions of the stomach produced no visible influence upon the gelatine in this respect during twenty days, after which the experiment was discontinued.¹

(2) In test-tubes with extracts from the stomachs fibrin dissolved during one to three days, and in the remaining it did not dissolve after twenty days, after which the experiment was discontinued.¹

(3) The casein test described also manifested a positive reaction only with extracts from the stomachs.

The results obtained from our experiments allow us to conclude that the stomach of the bee and drone contains a peptic ferment acting in an acid medium, whereas the crop, small intestine, and part of the large intestine with the rectal glands do not produce this ferment.

Trypsin.

For the determination of trypsin we employed:

(1) 10 per cent. alkaline gelatine, as in the case of pepsin; liquefaction of the gelatine followed after one to three days only in extracts from the stomachs.

¹ In order to test the sterility of liquefied gelatinous mixture and the fibrin solution sowing was made in agar, but during three days at 37° C. no growth was observed.

NO. 263
(2) Alkaline solution of casein. To 10 c.c. of 1 per cent. solution of casein containing 1 in 200 c.c. 10 drops of 10 per cent. solution of soda were added 2 c.c. of extracts of ferments tested; the test-tubes with the mixture were placed for one hour in water at a temperature of 38-40° C, after which a 0.5 per cent. solution of citric acid was added to their contents by drops evoking a turbidity in the solution of undigested casein.

By applying the methods described we obtained the same results in all cases, trypsin being established only in the stomach of the bee and drone, whilst the remaining parts of the intestine were devoid of it.

Chymosin.

Regarding the presence of chymosin in the organism of the bee there are no data in literature.

For its determination we used a mixture of 10 c.c. of milk + 90 c.c. of water + 1 c.c. of 10 per cent. calcium chloride.

To 10 c.c. of this mixture was added 1 c.c. of extracts of the ferment tested, and the liquid was placed in a water-bath at 40° C.

The extracts from the stomach always caused milk to coagulate; the formation of the coagulum in the experiments conducted being observed not earlier than after two, and not later than after fifteen minutes. In the test-tubes containing extracts from the remaining portions of the intestine the milk did not coagulate during three hours, after which the experiment was stopped.

Thus our experiments show that the stomach of the bee and drone contains abomasum ferment of considerable activity.

Emulsin.

For the determination of the ferments splitting the glucosides we used 1 per cent. solutions of amygdalin, salicin, and arbutin. The results obtained were negative. It was impossible at that time to purchase andromedotoxin, which produces the toxic properties of honey as well as any other glucosides.
TABLE X. SUMMARY TABLE OF FERMENTS ESTABLISHED IN DIFFERENT PORTIONS OF THE INTESTINE OF THE WORKER-BEE AND DRONE.

<table>
<thead>
<tr>
<th>Name of ferment</th>
<th>Crop</th>
<th>Stomach</th>
<th>Small intestine</th>
<th>Large intestine</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td></td>
<td></td>
<td>-</td>
<td>±</td>
<td>Found in the large intestine only during the second half of hibernation.</td>
</tr>
<tr>
<td>Amylase</td>
<td></td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inulase</td>
<td></td>
<td></td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactase</td>
<td></td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invertase</td>
<td></td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipase</td>
<td></td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lepsin</td>
<td></td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trypsin</td>
<td></td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chymosin</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Emulsin</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

+ denotes constant presence of ferment.
± denotes indefinite periods of ferment.
- denotes absence of ferment.

CONCLUSION.

At the conclusion we shall examine the distribution of ferments in the different parts of the ventriculo-intestinal tract of the bee, as adduced in the table above (Table X).

It is quite natural that digestive ferments should be found only in the stomach of the bee. The latter presents the mid-gut of this insect, i.e. that part of the digestive tract which is developed from the entoderm. Contrary to the fore- (=crop = honey-stomach) and hind-guts (small and large intestines) the stomach of the bee is devoid of an inner chitinous lining; it is true it is provided with a peripheral membrane (Pl. 16, fig. 9, p) clothing the food masses in the lumen of the stomach, but this membrane is secreted by the entodermal epithelium of the latter and differs in its properties from the chitinous cuticle, being soluble in hydrochloric acid. It is permeable to ferments, and, according to Petersen's experiments, even contains them in its own substance. The fact that ferments are produced only in the mid-gut of the bee is fully in accordance with the phenomena observed in other insects. In all arthropods the chief digestive processes take place in the mid-
gut and its derivatives (digestive glands—hepatopancreas) when such are present.

On account of the difference in the mode of life and nutrition between the worker-bee and drones, we examined and compared the intestinal ferments of both in the hope of tracing some points of difference between them. However, in the conditions of the experiments conducted, when the ferments were determined only qualitatively we failed in our attempts. It was impossible to discover any visible difference between the ferments.

An examination of the comparative table of ferments in the intestine of the bee adduced above reveals the fact of the inconstant presence of catalase in the extracts from the large intestine. This circumstance seems to produce a certain dissonance in the results of the work and disagrees with the generally accepted facts. However, on closer examination it is explained quite definitely and convincingly. As a matter of fact catalase was discovered in extracts from the rectum not accidentally but at a certain time of the year, viz. in spring, previously to the hives being removed from their hibernating quarters.

At this period catalase is abundant in the rectum, but already after the first flights of the bees its quantity decreases sharply and two days later it is already impossible to trace any of this ferment in the rectum. These facts can be naturally connected with the work of the intestine in winter. Bees feed all the time, but during the period of seclusion in the hive they do not excrete at all. The faeces accumulate in the rectum and distend it to extraordinary dimensions, as is represented in Plate 15 of figures. On comparing the intestine (drawn at the same magnification) of a bee that has hibernated previously to its discharge (fig. 3), with a bee dissected in summer in the usual conditions of its existence and activity, we may form a clear idea of the degree to which the intestine is overfilled. The stomach becomes shorter and thicker, especially large dimensions are attained by the rectum which assumes the aspect of an enormous ovoid bladder. Doubtless the accumulation and long retention of faeces in the rectum reflects
in one or another degree upon the process of metabolism in the bee. Such an accommodation is presented by the production of catalase in the rectum at a period when the bee is incapable of excretion. The increased production of this ferment stands in connexion with the demands of the organism for more energetic oxidative processes, in which there is not so much need in summer when the intestine of the bee discharges its excrements normally.

After we have convinced ourselves of the logical necessity of the presence of catalase in the rectum, we must solve the question regarding the place where this ferment is produced. Two possibilities may be discussed in this connexion: (a) either catalase is produced by the walls of the rectum in the bee (local origin of the ferment), or (b) catalase is transported to the rectum together with the food from the anterior portions of the intestine, namely, from the stomach.

We shall first discuss the latter possibility. In preparing extracts from the rectum the large intestine of the bees was cut; the faeces falling out themselves, the wall of the intestine washed repeatedly in a fresh physiological solution of common salt. Only after being thus cleaned of its contents the intestine was rubbed up with sand in glycerine. The measure of precaution described guarantee to a certain degree the purity of the extracts prepared, and therefore allow one to ascribe the property of catalase production to the walls of the rectum.

The conclusion set forth is indirectly corroborated by another circumstance. If catalase were transported into the rectum from the stomach with the digested food, we should expect this ferment to be present not only in the rectum (the hindmost portion of the intestine) but in the small intestine uniting the rectum with the stomach as well.

However, catalase was absent in extracts from the small intestine in all cases, notwithstanding the fact that they had been less carefully prepared. This part of the intestine is usually not removed from it, and the extracts were prepared from whole pieces of the small intestine with all its contents. Since the extract from the anterior portion of the intestine
and from its contents contained no catalase, whilst the extract from the wall of the posterior part of the digestive tract alone contains this ferment, the inevitable conclusion is that catalase is produced by the rectum itself in the bee, and is not brought there from other parts.

Now the question arises, in what part of the rectum is this ferment produced? In the first part of the work the structure of the rectum was described in detail, and it was mentioned that the latter differs in structure from the crop of the bee in the presence of eight elongated rectal glands. The wall of both rectum (Pl. 17, fig. 20, ep) and crop (Pl. 16, fig. 5, ep) is formed by flat epithelium bearing no characters peculiar to glandular tissue. Therefore it is difficult to ascribe to it the property of producing ferments; and, indeed, in the crop they are never produced. It may therefore be naturally concluded that the place where catalase is secreted is presented by the rectal glands in the plump epithelial cells of which are found granules of zymogen (Pl. 17, fig. 21, ep). The correctness of such a conclusion stands somewhat in contradiction to the fact that catalase may also be produced by non-glandular tissue. Thus this ferment is present in the nerve-tissue of some animals. In the near future we shall endeavour to solve the question discussed more precisely. In the dilated rectum of the bee it is possible to separate the anterior part with the rectal glands from the posterior consisting only of flat epithelium.

An investigation of the extracts from these parts of the rectum will, possibly, be able to give a definite answer to the question regarding the rôle of the rectal glands which have hitherto been mysterious organs in insects. Concerning their rôle only suppositions have hitherto been expressed. Berlese supposes that these glands serve to absorb the remains of food, and there may be also present some kind of valve for the retention of the contents of the intestine previously to the final formation of the faeces. N. A. Cholodkovsky (1912) thinks that it is possible to speak only of a sort of excretive function of the rectal glands, under the cuticle of which in Lepidoptera and the cricket (Gryllus domesticus) he observed an accumu-
lation of some kind of excretion. Vallé (1900) supposes that 'les papilles rectales des Diptères jouent deux rôles: le rôle respiratoire et le rôle sécréteur. Rôle respiratoire par les gros troncs trachéens et les petites ouvertures qui servent de débouchés aux ramifications trachéennes; rôle sécréteur par les cellules géantes et les pores terminaux leur donnant ouverture dans la cavité rectale' (loc. cit., p. 60).

Thus, Vallé unites the views regarding the glandular rôle of the rectal glands expressed by Lowne (1869) and regarding their analogy to the rectal gills of the dragon-fly larvae (Leydig, Chun, 1876).

It is quite possible that these organs discovered by Swammerdam in the bee play different rôles in insects. It is remark- able that the rectal glands are absent in beetles.

In other insects they appear only at the end of the pupal stage, and only the dragon-flies (Libellulidae) are provided with these glands in the larval stage as well (Faussek, 1887). Evidently these organs, the function of which is mysterious, stand in some connexion with the metamorphosis of insects.

On comparing these considerations with facts observed in bees its rectal glands may with a considerable degree of probability be regarded as glands one of the functions of which is the seasonal production of catalase.

Whereas the rectum of the bee is capable of producing a ferment albeit periodically (catalase), its crop lacks this property absolutely.

No ferments were ever established in extracts from the honey-stomach. This circumstance allows us to take a step nearer toward the solution of the question regarding the process of honey formation. The bee takes in the nectar into the crop from which it deposits it into the honeycombs. Does the crop present a passive reservoir adapted only to temporary conservation of the nectar, or does some other biochemical process, besides the splitting up of cane-sugar, take place in it? What takes place in the nectar deposited in the honeycombs during the ripening of the honey? In order to solve this question one of us (E. Zarin) had previously (1917) conducted
experiments by feeding bees chiefly on cane-sugar syrup which was successively passed through their organism twice.
In the first experiment the bees received twenty-five pounds of syrup; when they deposited it in the honeycombs two days later the honey taken out of them was again offered the bees; the honey deposited for the second time was left in the hive till the moment of sealing up, after which it was again offered to the bees; the honey deposited for the third time underwent chemical analysis similarly to the sugar, syrup, and honey of the first and second deposits.

When the honey ripens the cane-sugar is inverted; when this occurs a certain quantity of dextrin-like substances which do not reduce Fehling's solution are produced. 'Thus in natural honey, besides the non-sugars of plant origin, are also contained such that are produced by the organism of the bee, probably with the help of a special ferment.'

The sugar syrup offered to the bees was quite devoid of ferments, whereas in the deposited portions the presence of invertase and diastase was discovered; therefore the ferments named could have found their way into the honey only from the body of the bee. Catalase was absent in such artificial honey, whereas it is always present in natural sorts. Evidently catalase is brought into the honey from the nectar.

These data throw light on the nature of the ferments in the samples of honey investigated—some of them (catalase) are of plant, others (invertase and diastase) both of animal and plant origin.

If invertase and diastase are brought into the honey by the bee, the question arises—where are these ferments produced in its organism? The investigation conducted by us throws some light upon this question. The most simple supposition is that the ferments of honey are produced by the walls of the reservoir into which the bee collects the nectar. That is, however, not the case, since the walls of the crop are not endowed with glandular properties. It was impossible in any circumstances to establish any ferment in the extracts from the crop.
Evidently the ferments of honey penetrate into it from other portions of the digestive apparatus; such may be either the stomach of the bee in which invertase and diastase are actually produced, or the salivary glands. If we assume that the ferments of honey are derived from the stomach of the bee, allowance must be made for the possibility of a kind of exgurgitation, or their passage from the stomach into the crop, i.e. in a direction opposite to the normal course of food.

The anatomical data do not allow of forming such a supposition, as the bordering valve of the crop is provided with a long tubular valvule which prevents the usual contents, and therefore the ferments of the stomach as well, from penetrating back into the honey-stomach. If the existence of exgurgitation of ferments were possible, then not only invertase and diastase would pass into the crop, but also catalase, which is always secreted by the sides of the stomach. In this case it would have been also discovered in the analysed portions of the artificial honey out of sugar syrup described above. The fact that this was not observed serves to confirm the conclusion that the catalase of honey is of vegetable origin, there being no basis for admitting the possibility of an elective exgurgitation of ferments from the bee's stomach.

In this connexion it should be remembered that regarding the possibility of exgurgitation in bees the investigators differ in opinion. Some of them, as Schönfeld, believe that the brood-food of bees is discharged by the latter from the stomach, whilst others regard it as a secretion of the large salivary glands occupying the greater part of the volume of the head in the bee (Pl. 15, fig. 4, $\delta r_1$). Regarding the ferments of honey a supposition analogous to the latter can be made. The ferments are produced by the salivary glands of the bee swallowed together with the nectar into the crop and removed from there on deposition of the honey into the honeycombs. Such a conclusion appears to be the most correct, although it also bears the character of probability, since the digestive properties of the salivary glands are unknown. In other insects very strong
digestive ferments (proteolytic ferment) have been found in the saliva; they are certainly present in the bee as well, otherwise the presence of a complex system of salivary gland would be incomprehensible. The task at hand is to study their ferments, if only it will be possible to apply to them the method of preparation for the extraction of ferments, which presents great difficulties in the given case.

The data obtained by our work allow of an attempt to a partial solution of the question regarding the food régime of the bee and regarding the assimilation of different sorts of food by it. We shall meanwhile adduce a particular case. Petersen fed bees on oil emulsion in sugar solution and arrived at the conclusion that 'das meiste Fett, auch der normalen Nahrung, den Darm passiert, ohne gespalten oder resorbiert zu werden. Der feste Aggregatzustand des Kotfettes und seine Löslichkeit in Alkohol sind merkwürdig und lassen an einen reichlichen Gehalt von Fettsäuren denken' (loc. cit., p. 148).

The fact that we have constantly found lipase in the extracts from the stomach of bees refutes Petersen's view just quoted that fat passes through the stomach of the bee without splitting. Since the stomach contains a special ferment, it is obvious that it is produced for a respective purpose, i.e. in the given cases for the digestion of fat.

The next stage in the study of the digestive processes in bees should be experiments on preferential feeding of these insects with different nutritive substances conducted on a wide scale and the determination of the character of excretion and action of ferments in artificial conditions. The results of such an investigation are important not only from the theoretical but from the practical point of view as well. Bee-keepers have always to deal with the difficult task of artificial feeding of bees in winter, which frequently results in the appearance of dangerous diseases among these insects. When the conditions in which the intestine of the bee works are known, and a clear idea regarding the metabolism of its substances during the different seasons of the year is arrived at, it will be possible to solve the question regarding the methods of feeding it
The alimentary canal in the bee artificially instead of taking false steps and applying empirical methods, as hitherto practised.

One of us (E. Zarin, 1917) has already made some progress towards the solution by purely chemical methods of the question regarding the necessity of acidulating the winter food of bees, which is of practical importance and is applied by many practical bee-keepers.

It was proved that acid (0.1 per cent. citric) produces no useful action upon the inversion of cane-sugar and the ripening of honey, whereas acid ' added to the food in the amount of 0.3 per cent. produces a depression not only in the process of sugar inversion, but in all the other processes taking place in the honey-stomach of the worker-bee, as well as in the hive during ripening of the honey '. In the final conclusions these data ' disagree with the view prevalent among bee-keepers regarding the necessity of adding acid to the food '.

At the first opportunity we shall endeavour to continue our work in the direction mentioned, which is especially advantageous, since in it deep scientific problems are conveniently combined with the requirements of practical life.

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EXPLANATION OF FIGURES.

GENERAL MEANING OF LETTERS.

ab, abdomen. an, anal aperture. bg, connective tissue. bl, vesicular evaginations from the surface of cells of the stomach epithelium. c, chitinous cuticle. cb, suprapharyngeal nodules of the nerve-chain. cl, chitinous cuticle of the surface of the capitulum of the stomach valve. cmf, muscle (Leidig's) columns. cs, marginal chitinous fillet of rectal gland. cp, head. d, protoplasm. da, alveolar layer of weakly staining protoplasm under the chitinous cuticle of the rectal gland. dr, pharyngeal salivary glands of the bee. ds, salivary glands of the thorax (Hinterkieferdrüsen). dr, salivary glands of the thorax (Hinterkieferdrüsen). ds, bacilliform striated layer of protoplasm. ep, epithelium. g, granular contents of the stomach. gr, granules and inclusions in the protoplasm. h, cavity of rectal gland. i, crop. ia, fore-gut. ic, cavity of the crop. im, mid-gut. ip, hind-gut. it, small intestine. k, crypts of the stomach. m, muscles. m1, transverse (circular) muscles. m2, longitudinal muscles. m3, longitudinal exterior muscles of stomach valve. m4, circular muscles of the crop. m5, longitudinal muscles of the crop. mb, basilar membrane. mf, microfibrils. mp, Malpighian vessels. n, nucleus. ne, nerve. o, oesophagus. p, peritrophic membrane. pc, lumen of stomach valve. ph, pharynx. pm, perimysium of muscles. r, rectum (large intestine). rg, rectal gland. sm, syncytium in rectal gland. sp, sarcoplasm. th, thorax. tr, tracheae. v, stomach. wc, its vacuole with secretion. wa, exterior wall of rectal gland. wp, 'hairy' margin of the epithelium of the stomach. z, margins of the exterior wall of the rectal gland with tracheae between them.
EXPLANATION OF PLATES 15, 16, AND 17.

PLATE 15.

Fig. 1.—Ventriculo-intestinal canal of the worker-bee in summer. Large intestine of small size.

Fig. 2.—Same in drone. Stomach much longer than in the worker-bee.

Fig. 3.—Intestine of hibernating bee. Large intestine filled with faeces and therefore presenting the largest portion of the intestine in dimensions. The arrows denote the points at which the intestine was cut for the preparation of extracts from it. All the three figures were made with Zeiss' binocular microscope, ob. I 55, oc. 1.

PLATE 16.

Fig. 4.—Schematic longitudinal section of the body of the worker-bee with its organs of digestion. Combined, from two figures of Zander's monograph. The arrows on the left show the subdivision of the body into head, thorax, and abdomen, whereas the ones on the right denote the subdivision of the intestine into the fore-, mid-, and hind-guts. The subdivisions mentioned do not correspond one with another, as the fore-gut \((o, i)\) passes through the head, thorax, and part of the abdomen.

Fig. 5.—Transverse section of the crop on the level of the stomach valve. \(cd\), cuticle of the surface of the valvular capitulum; \(ic\), cavity of the crop. Haematoxylin; eosin. Zeiss; ob. AA, oc. 4.

Fig. 6.—Slightly oblique section of the stomach of the bee. Its cavity is filled with a very great number of peritrophic membranes \((p)\) disposed in concentric layers one on another. Zenker formol; Heidenhain's iron haematoxylin. Zeiss; ob. AA, oc. 1.

Fig. 7.—Part of transverse section of the stomach of the bee. A crypt in the depth of the epithelial fold is visible. Above the crypt is a vacuole with secretion. The hibernating bee was fixed in April. Duboscq's fluid, Mann-Holland stain. Zeiss; \(\frac{1}{2}\) hom. imm., oc. 0.

Fig. 8.—Part of the wall of the stomach in the bee, dissected in May. The section has passed obliquely, on account of which the cells of the folds appear to be set on slender peduncles and interrupted. Zeiss; ob. \(\frac{1}{2}\) hom. imm., oc. 1. Duboscq's fluid; Giemsa stain.

Fig. 9.—Transverse section of the wall of the stomach to show the formation and the rubbing off of the peritrophic membranes \((p)\) developing at the expense of the 'hairy' layer of the epithelial plasm \((wp)\). Above the crypt \((k)\) the vacuole displacing the 'hairy' layer of the plasm is situated. Zenker formol; Heidenhain's iron haematoxylin. Zeiss; ob. \(\frac{1}{2}\) hom. imm., oc. 1.

Fig. 10.—Peritrophic membranes in the cavity of the stomach of the
Fig. 11.—Part of the epithelial wall of the stomach in the bee dissected in May. Between the glandular cells is arranged the crypt, the cells of which also discharge a secretion accumulating in the globular vacuole (vc). The epithelial cells bear on their surface the 'hairy' layer of protoplasm (wp). Duboscq's fluid; Giemsa stain. Zeiss; ob. 1/2 hom. imm., oc. 4.

Fig. 12.—Part of the wall of the stomach of the same bee. In the 'hairy' layer of the epithelial cells are visible swellings of the superficial layer of protoplasm corresponding to the vesicular evaginations of the cells observed in the mid-gut of the larvae of Ptychoptera by Van Gehuchten. Mann-Holland stain. Zeiss; ob. 1/2 hom. imm., oc. 4.

Fig. 13.—Tangential section of the wall of the stomach in the bee. The rounded evaginations of the epithelium with cryptae at their bottom are visible in section. Duboscq's fluid; Giemsa's stain. Zeiss; ob. DD, oc. 4.

Fig. 14.—Longitudinal section of circular fibres of the muscular membrane of the stomach. sp, sarcoplasm. Giemsa’s stain. Zeiss; 1/2, oc. 4.

Fig. 15.—Longitudinal section of a fibre from the muscular membrane of the stomach of the bee; the abundance of sarcoplasm (sp) with nuclei in it (n) is visible (see fig. 16, Pl. 17). Haematoxylin, eosin. Zeiss; ob. 1/2 hom. imm., oc. 4.

Fig. 16.—Transverse section of muscle-fibres from the circular membrane of the stomach in the bee. The fibres are rich in sarcoplasm. The nucleus is disposed beyond the area occupied by the myofibrils. Haematoxylin; eosin. Zeiss; ob. 1/2 hom. imm., oc. 4.

Fig. 17.—Part of transverse section of epithelium of the small intestine of the bee. In the cells are visible vacuoles with secretion and the striated superficial layer of plasma (ds) covered with a chitinous cuticle (c). Zenker formol; Mann-Holland's stain. Zeiss; ob. 1/2, oc. 4.

Fig. 18.—Transverse section of the fibre of the muscular membrane of the small intestine in the bee. The fibre is thick; nuclei are disposed along the axis of the fibre surrounded exteriorly by tufts of myofibrils. The fibre is enveloped in the perimysium. Duboscq’s fluid; Heidenhain’s iron haematoxylin. Zeiss; ob. 1/2 hom. imm., oc. 4.

Fig. 19.—Rectal glands in the wall of rectum. Total surface preparation. Alcohol; borax carmine. Ob. Winkler 1, oc. 0.

Fig. 20.—Schematic structure of rectal gland. The drawing represents the wall of the rectum with half of the gland inserted in its wall. Rectum represented exteriorly.
Fig. 21.—Transverse section of rectal gland of a bee that has hibernated. In the cells of its epithelium are visible numerous granular inclusions and granules of secretion. Duboscq's fluid; Heidenhain's iron haematoxylin. Zeiss; \( \frac{1}{3} \) hom. imm., oc. 0.

Fig. 22.—Oblique transverse section of rectal gland of a summer bee. The network of deeply stained cell borders in which tracheae pass (fig. 25, Pl. 17) are visible. In the protoplasm of the epithelial cells there are no inclusions. Zenker formol. Same stain and magnification as in fig. 21.

Fig. 23.—Part of longitudinal section of the rectal gland. The entrance of the trachea between the cells of its external layer and the formation of lateral transverse branches of the trachea under the surface of the gland are visible. Zenker formol; iron haematoxylin. Zeiss; \( \frac{1}{2} \) hom. imm., oc. 4.

Fig. 24.—Network of tracheae under the surface of the inner wall of the rectal gland. Same treatment. Zeiss; \( \frac{1}{3} \) hom. imm., oc. 1.

Fig. 25.—Part of surface section of the exterior wall of the rectal gland. Tracheae running between the cells are visible. The surfaces of the latter adjacent to them stain sharply black. Same treatment and magnification as in preparation no. 23.

Fig. 26.—Cuticle of the epithelium of the small intestine with numerous bacteria on its surface. Zenker formol; Giemsa. Zeiss; ob. \( \frac{1}{2} \), oc. 4.
E. Pavlovsky, del.