EXPERIMENTS

FOR STUDENTS IN THE

HARVARD MEDICAL SCHOOL

Third Edition

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NOTICE

THE experiments performed by Harvard medical students are contained in the following publications:

1. AN INTRODUCTION TO PHYSIOLOGY, Parts I and II, containing experiments in general physiology, including muscle and nerve, and in the circulation of the blood.

2. AN INTRODUCTION TO PHYSIOLOGY, Part IV, Physiological Optics.

3. EXPERIMENTS FOR STUDENTS IN THE HARVARD MEDICAL SCHOOL, Third Edition, containing experiments upon the central nervous system, skin, general sensations, taste, vision, fermentation, digestion, blood, respiration, and metabolism.

Additional experiments will be added as rapidly as possible.

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EXPERIMENTS

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I

THE CENTRAL NERVOUS SYSTEM

Simple Reflex Actions

The Spinal Cord a Seat of Simple Reflexes. — 1. By means of a hook or thread passed through the lower jaw suspend vertically a frog the brain of which has been destroyed with the seeker; the legs must not touch the table. Pinch a toe with the forceps.

The leg will be drawn up.

A stimulus to the skin has caused the contraction of muscles. The afferent impulse set going by the sensory stimulus is changed into a motor efferent impulse. This is an example of *reflex action.*

2. Destroy the spinal cord with the seeker. Stimulate the skin of the right leg electrically and mechanically.

In no case will the sensory stimulus call forth the reflex contraction of a skeletal muscle. Yet the nerves coming from the skin and going to the muscles are still intact. Only the spinal cord has been destroyed.

The conversion of sensory into motor impulses for skeletal muscles is a function of the central nervous system.

Influence of Afferent Impulses on Reflex Action.—Destroy the brain of a strong frog with the seeker. Gently pinch a toe of the right foot.

Only the right leg will be drawn up.

Pinch a toe of the left foot.

Only the left foot will be drawn up.

Pinch a finger.

Only the corresponding arm will move.

Pinch the whole foot sharply.

More extended movements will be made.

The character and location of the stimulus affect the resulting contraction.

Threshold Value Lower in End Organ than in Nerve-Trunk.—1. Carefully expose the sciatic nerve. Determine the least strength of tetanizing current that will cause a crossed reflex when applied to the skin of the foot. Now apply the same stimulus to the trunk of the nerve.

As a rule, the intensity required to produce reflex action is less when the stimulus is applied

to the peripheral endings of the sensory nerves than when the nerve-trunks are stimulated.

2. Divide the skin over the back in the median line. Raise the skin on one side until the small nerves which pass across the dorsal lymph sac to innervate the skin come into view. Sever from the surrounding skin a piece about one centimetre square containing the endings of one of the nerves. Let the isolated piece with its nerve endings remain connected with the body only by the trunk of the nerve. As before, determine the least strength of tetanizing current that will cause a reflex movement when applied to the nerve-endings in the skin and to the nerve-trunk respectively.

The threshold value for reflex action will again be found lower in the nerve-endings than in the nerve-trunk.

Summation of Afferent Impulses. — Pass two fine copper wires about the frog's foot a centimetre apart and connect them with the secondary coil. Connect the primary coil through a simple key with a dry cell. Stimulate with regularly repeated make induction currents of such strength that single stimuli cause no reflex contraction.

Summation of the subminimal stimuli will finally cause reflex contraction.

Determine that the number of stimuli neces-

sary to produce a reflex becomes smaller when (1) the strength of the induction currents is increased, and (2) when the interval between the stimuli is lessened.

Segmental Arrangement of Reflex Apparatus. — 1. Gently pass the seeker over the abdominal walls on one side.

The muscles in that region only will twitch.

Repeat the stimulus, but use a stronger pressure.

The area contracting will increase in extent approximately in proportion to the increase in the stimulus. The afferent nerves from any one region are more closely related to the efferent nerves of that same region than to those of other regions. The fact that both afferent and efferent fibres spring from the cord at the same level suggests that their nerve cells lie also at approximately the same level. On increasing the stimulus the afferent impulse spreads from segment to segment of the cord. Further evidence of the segmental arrangement will be gained by the following experiment.

2. With a clean, sharp knife make transverse sections of the spinal cord, beginning in the cervical region. A short time after each section test the reflexes from the hind limb by mechanical stimulation.

Note the level below which no section can be made without rendering the reflex impossible. The nerve cells concerned in this reflex lie on the caudal side of this line.

Now in a second frog make transverse sections, beginning at the caudal end of the cord, and test the reflexes as before, until the level is reached beyond which a section will destroy the reflex.

Observe that the portion of the cord comprised between the two levels determined forms a segment which contains the central apparatus concerned in the reflex studied.

Reflexes in Man. — 1. *From the Skin.* — Rub the plantar surface of the foot gently with some hard object.

The foot will be retracted reflexly.

Similar results may be obtained by rubbing the skin of the inside of the thigh, which will cause contraction of the cremaster muscles; or by rubbing the skin of the abdomen, which will be followed by contraction of the abdominal muscles.

These reflexes are of importance in clinical diagnosis because by means of them the seat of a diseased area in the central nervous system may sometimes be defined, since the reflex depends on the integrity of the corresponding reflex arc.

2. *Cornea Reflex.* — Touch the cornea gently with a thread.

The eye will be closed involuntarily.

3. *Throat Reflex.* — Touch the posterior wall of the throat.

The movements of swallowing will usually follow.

4. *Pupil Reflexes; Light Reflex.* — Close one eye for several seconds, then open it quickly.

Note the contraction of the pupil.

5. *Consensual Reflex.* — Close one eye as before, but watch the pupil of the other eye when the first is opened again.

The pupil will contract.

6. *Accommodation Reflexes.* — Look alternately at a near and a far object. The pupil will contract when the eye adjusts itself to see the near object.

Tendon Reflexes

Knee Jerk. — Sit in such a position that the knee is bent at a right angle, and the foot hangs free. Let an assistant strike the patellar ligament with the side of the hand.

Note the sudden contraction of the extensors of the thigh, the so-called knee jerk.

Flex the knee at different angles and determine in which position the resulting contraction is greatest.

Knee jerk can be obtained only within certain limits of extension.

Let the subject immediately before the stimulus is applied forcibly contract some other group of muscles; clench the hand, for example.

The knee jerk is reinforced.

Ankle Jerk. — Bend the foot at right angles to the leg, and strike the tendo Achillis. The experimenter should hold the end of the foot in his left hand.

Contraction of the gastrocnemius muscle will be observed.

Gower's Experiment. — Strike the side of the tendo Achillis.

A contraction will result.

Support the other side of the tendon so that the gastrocnemius muscle will not be stretched by the blow. Repeat the experiment.

No contraction follows. The tendon jerk requires for its production a rapid stretching of the muscles involved in the contraction.

Try to obtain tendon jerks from other muscles; for example, the triceps humeri, flexors of hand, and masseter muscles.

Normally no response will be obtained.

The experiments are of value in diagnosis of diseases of the central nervous system.

Effect of Strychnine on Reflex Action

Inject with a glass pipette a few drops of 0.5 per cent solution of sulphate of strychnine into the dorsal lymph sac of a frog the brain of which has been destroyed with a seeker.

After a few minutes, very weak afferent impulses will be sufficient to call forth general spasmodic reflex actions. Note that (1) the strychnine reflexes are paroxysmal, (2) the muscles fall into more or less prolonged rigidity (tetanus), and (3) the extensors overcome the flexors, the limbs being strongly extended.

The characteristic action of strychnine is evidently not dependent on the brain.

Destroy the spinal cord with a seeker.

Stimulation of muscles and nerves will not cause spasmodic contractions.

Strychnine acts on the spinal cord, but not on the muscles or the peripheral nerves.

Complex Co-ordinated Reflexes

Removal of Cerebral Hemispheres. — Place a frog under a glass jar containing a small sponge wet with ether. Be very careful not to kill the frog. When insensibility is complete, place the animal on a frog-board. Cut through the skin in the median line of the skull, from the nose to the

vertebral column. Connect the front margins of the two tympanic membranes by a transverse incision through the skin. This transverse line will pass over the junction of the cerebral lobes with the optic lobes. Strip off the parietal bones with forceps, beginning at the anterior end opposite the anterior margin of the orbit. When the cerebral hemispheres are uncovered, they may be removed from before backwards. Avoid injuring the optic lobes. Work rapidly but carefully. If the ether effect diminish before the operation be finished, replace the frog under the glass jar for a few moments. As soon as the hemispheres are removed, sew up the wounds in the skin.

Note the signs of profound inhibition.

If the operation be done carefully, the shock will gradually pass away, and the functions possible in the absence of the cerebrum may then be determined. Put the frog aside, moistening his skin occasionally, but not otherwise disturbing him, and prepare a second frog for the experiment upon the "croak reflex" (page 10). When this operation is completed, resume the observations on the first frog, while the second frog recovers from the shock.

1. **Posture, etc.** — Write down the differences between the frog from which only the cerebral hemispheres have been removed and a frog in

which the whole brain has been destroyed with the seeker, in respect to posture, power to regain feet when laid on back, respiratory movements, position of eyelids, leaping and swimming.

2. **Balancing Experiment.** — Place the frog on a somewhat roughened board, about 20 inches long, 8 inches wide, and 1 inch thick. Tilt the board gradually.

The frog remains motionless until his centre of gravity is disturbed. He then moves forward in an attempt to reach a stable position. By careful management, he can be made to climb up the inclined board, perch upon the narrow edge, and, the board still turning, descend head-first on the opposite side.

3. **Retinal Reflex.** — Place the frog deprived of cerebral hemispheres in front of a bright light; for example, an incandescent electric lamp. Interpose some object, such as a small instrument case, between the light and the frog, so that a strong shadow is cast upon the frog's eyes. Stimulate the frog by pinching the skin of the back.

The frog will jump, but will avoid the object which casts the shadow.

4. **Croak Reflex.** — Sever the large hemispheres from the remainder of the brain of another frog by passing a knife through the cranium to the

base of the skull from side to side in a line joining the anterior margins of the tympanic membranes. (Where possible, a male frog should be selected for this experiment. Males may be recognized by the cushion-like thickening on the innermost digit of the manus, or hand; the male Rana esculenta possesses bladder-like, resonating pouches connected on each side with the mouth cavity.) After the immediate shock of the operation has passed, stroke the back over the anterior half of the spinal cord.

Reflex croaking will be observed.

The croak reflex can be inhibited by simultaneous pinching of one of the limbs or other strong stimulation. (Compare page 15.)

If the experiments on the frog in which the cerebral hemispheres were extirpated were not satisfactory, repeat them on this frog in which the hemispheres were simply separated from the remainder of the brain.

These observations teach that very complicated co-ordinated actions are possible in the absence of the large hemispheres. Only simple reflexes are possible when the whole brain is removed. Consequently, the seat of these complicated reflexes must lie in the brain between the cord and the cerebral hemispheres.

Apparent Purpose in Reflex Action

1. Destroy the brain of a frog with the seeker. Dip small pieces of filter paper in strong acetic acid. Remove the superfluous acid, lay the paper bearing the acid on (1) the frog's thigh, (2) the foot, (3) the back. After each stimulation note the character of the reflex movement, and then carefully wash the acid from the skin.

The movements are related to the areas stimulated in a certain purposeful way. Efforts are made apparently to brush away the acid paper.

2. Place the acid on the flank of the right leg. Usually the leg stimulated strives to brush away the paper. Hold this leg fast.

The other leg (the left) will be used to remove the acid from the opposite limb. (This experiment succeeds best in strong, lively frogs.)

3. Place an uninjured frog in an evaporating basin containing sufficient water to immerse the frog to the neck and covered with wire gauze to keep him from jumping out. Warm the water.

As the temperature rises to from 20°–30° C. the frog will attempt to escape.

Repeat the experiment with the frog the brain of which has been destroyed.

No movements of escape will be noticed.

About 35°, muscular twitchings will be seen. At 38°–40° death takes place and the muscles become rigid (heat rigor).

This observation shows that volition in all probability is absent in the brainless frog. It follows that reflex actions are not volitional; their "purpose" is only apparent.

Reflex and Reaction Time

Reflex Time. — Destroy the brain of a frog with the seeker. Hold one leg of the frog aside with the glass rod. Bring beneath the other a small beaker almost full of dilute sulphuric acid (2:1000). Raise the beaker until the foot is immersed to the ankle. Count the seconds between the application of the stimulus (sulphuric acid) and the withdrawal of the foot.

This interval is the reflex time.

Wash the foot carefully in the bowl of water.

Reaction Time. — Smoke a drum. Raise the drum off its friction bearing by turning the screw at the top of the shaft. Place the writing point of an electromagnetic signal against the smoked paper. Arrange a tuning fork to write its curve near that of the signal. Connect the signal through two simple keys and a dry cell with the primary coil of an inductorium arranged for maximal single induction currents (posts 1 and

2). Let stimulating electrodes pass from the secondary coil (bridge up) to the tongue of the subject. Let the subject hold one key closed until he feels the stimulus on the tongue.

Direct the subject to shut his eyes. Let the observer start the tuning fork, spin the drum, and stimulate the subject by completing the primary circuit. The instant the subject perceives the stimulus, he will break the circuit by releasing his key. By means of the tuning fork curve determine the interval between stimulation and response. This interval is the reaction time plus the errors of observation; for example, the latent period of the electromagnetic signal. Repeat the experiment three times and take the mean of the results.

In the laboratory note-book make a list of the links in the chain between stimulus and response, and state as far as possible the errors of observation.

Reaction Time with Choice. — Connect the side cups of a pole-changer (without cross wires) to the posts of the secondary coil. Connect one pair of end cups with the usual stimulating electrodes, the other pair with large brass electrodes covered with wet cotton. Let the ordinary electrodes touch the forehead, the other pair the hand of the subject. The other connections should re-

main as before. Repeat the preceding experiment but tell the subject to signal only when the tongue (or hand) is stimulated. In order to do this he must add to his former reaction a decision as to the part stimulated.

Reaction time with choice is longer than simple reaction time. In general, the more complicated the mental processes involved, the longer will be the reaction time.

Inhibition of Reflexes

Through Peripheral Afferent Nerves. — Expose the left sciatic nerve for a distance of about 15 mm. in a frog the brain of which has been destroyed. Tie a thread around the distal end, and sever the nerve at the peripheral side of the ligature. Place the central stump of the nerve on the electrodes of the inductorium, the short-circuiting key being closed. Make the primary circuit, and set the hammer vibrating. Now open the short-circuiting key, bring the right foot of the frog into the dilute sulphuric acid up to the ankle, and count the seconds from the moment of immersion to the moment of withdrawal, continuing meanwhile the stimulation of the central end of the left sciatic nerve.

The latent period will be much prolonged.

Wash off the acid carefully.

Reflex actions may be inhibited by the simultaneous stimulation of sensory nerves.

Through Central Afferent Paths; the Optic Lobes. — 1. Expose the brain according to the directions already given (page 8). Immediately posterior to the cerebral hemispheres lie the optic lobes, two gray spherical bodies. Separate the cerebral hemispheres from the optic lobes by a transverse incision, and carefully remove the hemispheres. Wait until the shock of the operation has passed. Now suspend the frog so that the tips of the toes hang above a shallow dish containing water made strongly sour to the taste with dilute sulphuric acid. Determine the reflex time. Wash off the acid and, after a moment's rest, sprinkle a very little finely powdered common salt on the cut surface of the optic lobes. Again determine the reflex time.

The reflex time will be found to be markedly increased by the stimulation of the optic lobes.

2. Prepare a second frog in the same manner. Determine the reflex time. Now instead of stimulating the optic lobes, remove them, and again determine the reflex time.

The removal of the optic lobes shortens the reflex time.

The Roots of Spinal Nerves

Destroy the brain of a strong, large frog with a seeker. Divide the skin over the vertebral column from the upper end of the urostyle to the level of the fore limbs. Hook back the flaps of skin. Remove the longitudinal muscles on either side of the spines of the vertebræ, thus exposing the bony arches. Saw through the arches of the 8th, 7th, and 6th vertebræ (there are ten vertebræ in the frog, counting the urostyle) in the order named. Clear away the bone and the underlying tissues until the last three or four pairs of roots shall be plainly seen. Grasp the filum terminale and cautiously lift the cord until the spinal nerve roots are clearly displayed.

The anterior roots are hidden by the large, superficial posterior roots. The conspicuous posterior root which seems to be the last is, in reality, the 9th, the next to the last; the last, or 10th, is smaller and lies close to the filum terminale. Place a silk ligature about the middle of an anterior and a posterior root on the right side. With single induction currents as stimuli observe that (1) the stimulation of only the central end of the posterior root calls forth a (reflex) movement, and (2) the stimulation of only the peripheral segment of the anterior root causes movement.

On this same side cut all the posterior roots.

No stimulus applied to the right leg will now discharge a reflex action. But stimuli applied to sensory nerves elsewhere may still cause reflex movements of the right leg. Motor impulses still pass to these muscles. But only the anterior roots remain.

Hence the anterior roots of spinal nerves transmit motor impulses from the spinal cord towards the muscles (efferent impulses); the posterior roots transmit sensory impulses from sensory surfaces towards the spinal cord (afferent impulses.)

Ludwig's Demonstration. — Destroy the brain of a large frog with the seeker. Remove the thoracic and abdominal viscera, taking care not to injure the sciatic nerve plexus. Remove the 7th and 8th vertebræ, taking the greatest pains not to injure the nerve roots. Divide the body transversely at this level, so that the anterior and posterior halves shall remain connected only by the anterior and posterior sciatic roots. Keep the roots moist with normal saline solution.

Demonstrate again that the anterior roots transmit efferent, and the posterior roots afferent impulses.

Localization of Movements at Different Levels of the Spinal Cord. — Separate the three roots which form the sciatic nerve. After tying a thread

about each root sever it from the spinal cord by a cut on the proximal side of the thread. Stimulate each nerve with a very weak tetanizing current. Note the different results obtained from nerves arising at different levels of the cord. Stimulation of the most anterior root causes marked flexion of the limb; stimulation of the middle roots, extension and internal rotation; and of the most posterior, simple extension.

In a frog whose nerves have not been cut expose the spinal cord and stimulate it at different levels in both directions along its length. The various movements of the hind limbs are localized at different levels of the cord.

Distribution of Sensory Spinal Nerves

Destroy the brain of a large frog with the seeker. Expose the lower half of the spinal cord by the method already described. On one side cut the dorsal sensory root of the 8th spinal nerve and on the other cut the sensory root of the 7th, 9th, and 10th. After the section of each root test the cutaneous sensibility of the limbs by placing upon the skin small pieces of filter paper (two mm. square) moistened, not dripping, with 0.2 per cent sulphuric acid. Make a map of the anæsthetic areas in each leg, and note the lack of correspondence.

Many skin areas are supplied by fibres from at least two sensory roots. The fields of distribution overlap.

Muscular Tonus

Brondgeest's Experiment. — Fasten a lightly etherized frog back uppermost on the frog-board. In a line between the ilium and the coccyx open the pelvic cavity by cautiously dividing the skin, fascia, and muscle. Divide the sciatic nerve roots on the operated side. Pass a hook or thread through the jaw and hang the frog up.

Observe that the limb the nerves of which have been cut is relaxed, so that the toes hang lower than those of the limb which still retains its connection with the central nervous system.

Apparatus

Normal saline. Bowl. Towel. Pipette. Stand. Muscle clamp. Bent hook. Inductorium. Dry cell. Electrodes. Large brass electrodes. Cotton. Key. Frog-board. Fine copper wire. One-half per cent solution of strychnine sulphate. Glass jar with ether and sponge. Balancing board. Strong acetic acid. Filter paper. Evaporating basin. Wire gauze. Bunsen burner. Thermometer. Dilute sulphuric acid (0.2 per cent). Beaker. Kymograph. Electromagnetic signal. Tuning fork. Pole-changer. Vertebral saw.

II

THE SKIN

Sensations of Temperature

Hot and Cold Spots.—With a lead-pencil point carefully explore an area about an inch square on the back of the wrist or hand. Mark with black ink the places where a distinct sensation of cold is felt, and with red ink those where the sensation is one of warmth.

The places indicated are the so-called hot and cold spots.

Outline. — Attempt to define more exactly the outline of one of the cold spots.

The spots are of irregular shape, — blotches rather than points.

Mechanical Stimulation. — 1. Gently tap one end of a small wooden rod the other end of which is placed on a well-defined cold spot.

The mechanical stimulation of the cold spot will give a sensation of cold.

2. Stimulate a warm spot mechanically.

Chemical Stimulation. — Rub a menthol pencil over a small area on the back of the hand.

A sensation of cold will be perceived. This is due to chemical irritation of the cold spots. The temperature of the area does not fall.

Electrical Stimulation. — It has been found that the stimulation of a well-defined cold or warm spot with moderately strong induced currents causes a sensation of cold or warmth respectively.

Temperature After-Sensation.—Stimulate a cold spot mechanically with a pencil point. Remove the point.

The sensation of cold outlasts the stimulus.

Balance between Loss and Gain of Heat. — Provide three beakers of water. Heat them to 20°, 30°, and 40° C., respectively. Place a finger of one hand in the water at 20°, and a finger of the other hand in the water at 40°. After the respective sensations of cold and warmth have disappeared, place both the fingers in the water at 30°.

The finger from the cold water will seem warm and that from the warm water cold. The temperature of the skin equals the balance between its heat loss and heat gain. When this temperature is raised or lowered, the warm spots or cold spots respectively are stimulated.

Fatigue. — Provide three beakers containing water at 10°, 32°, and 45° C. respectively. Place a finger of one hand in the beaker at 32°, and a

finger of the other hand in the beaker at 45°. After 45 seconds place both fingers in the water at 10°.

The finger taken from the water at 32° (which is about the normal temperature of the hand) will *feel* colder than the other finger. Extreme temperatures of heat or cold fatigue the temperature spots.

Relation of Stimulated Area to Sensation.—Insert a finger of one hand in a beaker of warm or cold water. Note the sensation. Insert a finger of the other hand in the water.

The intensity of the sensation will increase with the extent of the surface stimulated.

Perception of Difference. — Provide two beakers of water, one at 30°, the other slightly warmer or colder. By introducing a finger first into the one and then into the other, and varying the temperature of the water, ascertain how small a difference in temperature can be detected.

Usually a difference of 0.5° C. is easily recognized.

Relatively Insensitive Regions. — 1. Compare the temperature sensation perceived on touching with a pencil point the median line of the forehead, nose, and chin with that perceived on touching the skin on either side of the median line.

The skin in the median line of the body is comparatively insensitive to temperature variations.

2. Similarly compare the mucous membrane with the skin.

The mucous membranes are much less sensitive than the skin.

Sensations of Pressure

Pressure Spots. — Explore the surface of the forearm by bringing the blunted point of a needle gently in touch with the skin.

At certain spots a distinct sensation of contact will be perceived. Other spots will give only dull sensations. Pressure, like heat and cold, is appreciated by scattered sense-organs in the skin, not by diffuse general sensation.

Note the relation of the pressure points (1) to the hair follicles, and (2) to the warm and cold spots mapped out in previous experiments.

Threshold Value. — Take from the human head several straight, strong hairs. Cement each to the end of a little stick of soft pine to serve as a handle. Provide a special lever, made as follows: With a hot pin burn a small hole at the middle of a straw about 25 cm. in length. Pass a needle through this hole into a cork held in the muscle clamp. Press the free end of the hairs

against different parts of the skin of the hand, arm, and face. Select hairs which when pressed against the skin of the respective regions give no sensation of pressure. Shorten the hairs until the pressure is just perceptible. This will be the "pressure threshold." Make a loop in a short silk thread and pass the loop about the lever exactly one millimetre from the axis. Hang on the end of the thread a light bent hook. Counterpoise the lever very exactly, so that the slightest force applied to the end of the straw will raise the lever from the after-loading screw. By counterpoising in this way, the lever becomes a balance. On the bent hook hang a ring of German silver wire weighing one decigram (0.1 gram). Find a point on the lever 100 mm. from the axis. The weight of one decigram suspended 1 mm. from the axis of the lever will be raised by a force of $\frac{1}{100}$ of a decigram, equal to one milligram (0.001 gram) applied 100 mm. from the axis. At 50 mm. from the axis, 0.1 gram, suspended 1 mm. from the axis, will be lifted by a force of $\frac{1}{500}$ gram (0.002 gram). Find the distance from the axis at which each testing-hair, when pressed vertically against the lever, will just fail to lift the lever; in other words, the point at which the pressure will be just sufficient to bend the hair. The number of millimetres

between this point and the axis of the lever, multiplied by one-tenth, will give the bending pressure of the hair in the fraction of a gram. Make ten observations on each hair and mark the mean bending value on the wooden handle.

Touch Discrimination. — 1. Close the eyes and let an assistant test the different parts of the skin of the hand, arm, and face for discriminating power. For each test separate the points of the æsthesiometer until they can be felt as two (ordinary drawing dividers or compasses can be used for an æsthesiometer).

Record your results in millimetres for finger-tips, palm of hand, back of fingers, back of hand, back of wrist, flexor and extensor surfaces of forearm, forehead, cheeks, lips, and tongue.

2. Separate the points of the æsthesiometer about 20 mm., and draw them gently side by side along the extensor surface of the forearm from the elbow to the wrist. Repeat the experiment on the flexor surface. Try the same for the cheek and lips, beginning near the ear and drawing the points so that one shall go above and the other below the mouth.

Describe the sensation in each case, and suggest an explanation.

Weber's Law. — Place the hand palm upward in a comfortable position on the table. Close

the eyes. Let an assistant place on the last phalanx of the middle and index fingers a small round box containing ten small shot.

When the subject has formed a clear perception of the weight, let an assistant add or subtract shot, and record the number of shot corresponding to the smallest difference in weight perceived by the subject (whose eyes of course should be kept closed). Repeat the experiment with 20, 30, 40, and 50 shot in the box respectively. Determine in each instance the ratio of the number of shot added or subtracted to the number with which each experiment was begun.

This ratio will be approximately constant. The degree of stimulation necessary to cause the perception of difference always bears the same ratio to the degree of stimulation already applied. Weber's law is less true for very small and very large weights than for those of medium value. It is a general law and holds good for visual judgments, etc.

After-Sensation of Pressure. — Place a rubber band about the head and allow it to remain for several minutes.

On removing the band, a distinct after-sensation of pressure will be felt.

Temperature and Pressure. — Place on the back of the hand supported on the table a coin the

temperature of which has been made such that it feels neither warm nor cold. Compare the pressure sensation (apparent weight) of this "normal" coin with that of similar coins warmed and cooled.

The hot or cold coin will seem heavier than the "normal" coin of equal weight.

Touch Illusion; Aristotle's Experiment. — Cross the right middle finger over the right index finger and place them on the palm of the left hand. Place a small shot between the crossed fingers in such a way that it shall touch the ulnar side of the middle finger and the radial side of the index finger. Roll the shot in the palm of the hand.

A sensation of two objects will be felt.

Apparatus

Black and red ink. Small wooden rod. Menthol pencil. Inductorium. Dry cell. Electrodes. Key. Three beakers. Stand. Ring. Wire gauze. Bunsen burner. Thermometer. Needle with blunted point. Muscle lever. Gram and ten-gram weights. German silver ring weighing 0.1 gram. Silk thread. Four small wooden handles for pressure-hairs. Bent hook. Drawing dividers (as æsthesiometer). Small round box containing at least 50 shot. Rubber band large enough to go around the head.

III*

GENERAL SENSATIONS

TICKLE

Irradiation. — Gently touch the skin near one nostril with a dry camel's-hair brush.

Note (1) the strong sensation produced by the slight stimulus; (2) the irradiation beyond the spot stimulated.

After Image. — Repeat the stimulus of the preceding experiment.

Measure in seconds the time during which the sensation outlasts the stimulus (after image).

Topography. — Test the tickle sensation at various points on the skin of the face, hands, and forearms. Determine whether the sensation is greatest about the several openings, where skin joins mucous or serous membranes; *e. g.*, the nostrils, the conjunctival sac, the auditory canal. Do the results indicate a protective mechanism?

Summation. — In one of the sensitive areas found in the preceding experiment determine the difference between the response to a single stimulus and to successive stimuli.

Fatigue. — In any sensitive area determine (1) the quickness with which the apparatus for the sensation of tickle is fatigued; (2) the duration of fatigue.

Pain

Threshold Value. — Arrange an inductorium for tetanizing currents. Place the electrodes on the tip of the tongue, and move the secondary toward the primary coil until no farther movement can be made without causing the stimulation to become painful. Determine for this region and for others of the mucous membrane of the mouth and of the skin what distance of the secondary coil from the primary separates the stimulus at which pain is just perceived from that at which the pain is distinct.

Latent Period. — In several individuals measure approximately the interval between the application of the stimulus (single break shock) and the resulting painful sensation.

Summation. — Determine the number of subminimal stimuli necessary to produce pain.

Topography. — Map upon the skin of the face and arm the areas specially sensitive to pain.

Individual Variation. — Compare the reactions of several individuals, and note the differences in threshold value, latent period, summation, and topography.

Temperature Stimuli. — Fill two bowls or large beakers with water twenty-five degrees respectively, hotter and colder, than the temperature

of the hand. Determine whether the increase or the corresponding decrease in temperature is the more painful to the immersed hand.

Motor Sensations

Judgment of Weight. — Lift the same weight twice, at first very slowly and then quickly.

The weight will appear lighter when raised quickly.

Sensation of Effort. — "Hold the finger as if to pull the trigger of a pistol. Think vigorously of bending the finger, but do not bend it.

"An unmistakable feeling of effort results.

"Repeat the experiment, and notice that the breath is involuntarily held, and that there are tensions in other muscles than those that would move the finger." (*Sanford.*)

Sensation of Motion. — Let the forearm and hand rest upon a table. Bring the four fingers of the hand together, and turn the hand so that it shall rest upright upon the ulnar side of the little finger. Close the eyes. Abduct the first finger.

The second, third, and fourth finger will seem to move in a direction opposite to the movement of the first.

IV

TASTE

Threshold Value. — Prepare solutions of cane sugar of the following strengths: 1 : 1000, 1 : 800, 1 : 600, 1 : 400, 1 : 200, 1 : 100. Take half a teaspoonful of the weakest solution into the mouth, roll it upon the tongue, and swallow it. Note whether a sweet taste can be perceived. Rinse the mouth thoroughly. Proceed with solutions of increasing strength until the sweet taste is just perceptible.

Topography. — 1. Select a solution of sugar slightly more concentrated than that just perceived to be sweet. With a small camel's-hair brush apply this solution to the several parts of the tongue and the palate. Determine the regions sensitive to taste. The mouth must be rinsed frequently. 2. Dry the upper surface of the tongue with a handkerchief. With a finely pointed camel's-hair brush apply a twenty per cent sugar solution to the individual fungiform papillæ and to the mucous membrane between them. Determine whether only the papillæ perceive taste.

Relation of Taste to Area stimulated. — Swallow a very small quantity of a minimal solution of sugar, as determined in the experiment upon

threshold value. Rinse the mouth, and then swallow a much larger portion of the solution.

The taste will be perceived more strongly, the larger the area stimulated.

Electrical Stimulation. — 1. Connect two small zinc electrodes through a simple key to a battery of four dry cells. Apply one electrode to an indifferent region, the other to the tongue. Close the key.

Note the sour taste at the positive pole and the alkaline taste at the negative.

V

VISION

Mapping the Blind Spot. — Fasten a rod fifteen inches from the table. Beneath the rod place a well-lighted sheet of white paper (a page of the laboratory note-book will serve). Make a small black cross near the left margin. Rest the chin upon the rod in such a way that the right eye shall look directly down at the cross. Place the hand over the other eye. A straw bearing a black pin-head will be drawn by an assistant from the cross along the horizontal meridian toward the temporal side of the eye under observation. The assistant will mark the point where the black object ceases to be visible, and the point at which it reappears. These are

the boundaries of the blind spot of the right eye in the horizontal meridian. Determine the boundaries in other meridians. Obtain similarly the outlines of the blind spot of the left eye.

Yellow Spot. — Close the eyes for half a minute, and then look at the clear sky or a brightly lighted surface through a solution of chrome alum in a glass bottle with parallel sides. The yellow spot will appear rose-colored in the blue-green-red solution. The yellow pigment absorbs some of the blue and green rays. The remaining rays form rose color.

Field of Vision. — Fasten in a vertical position a sheet of white paper about 50 cm. high and 60 cm. broad. (It may be pinned to the wooden stand set on edge upon the electrometer box.) About 20 cm. from the left margin and 30 cm. from the lower margin of the paper mark a small cross. Let the subject rest his chin upon a rod clamped to the iron stand in such a way that the right eye shall look directly at the cross. Cement the squares of black, red, green, and blue papers to the ends of separate straws. Carry the black square from without inwards along the horizontal meridian intersecting the cross. Mark the point at which the black object enters the field of vision. This point is the temporal boundary of the visual field in the

horizontal meridian. Determine in the same way the boundary on the nasal side. Repeat for several other meridians. A line joining the points obtained will bound the visual field.

Determine the visual field for red, green, and blue. Always pass the test color from without inwards. The subject should be ignorant of the color to be used, and should name the color as soon as it enters his visual field.

Color Blindness

The three large skeins show the test colors.

1. *Light Green.* — Palest (lightest) shade of very pure green, — neither yellow-green nor blue-green to the normal eye. Light green is chosen because, according to the Young-Helmholtz theory, it is the whitest of the colors of the spectrum, and, consequently, is most easily confused with gray. Light shades are employed because it is difficult to distinguish between strongly illuminated shades.

2. *Purple* (*Rose*). — A skein midway between lightest and darkest purple. Chosen because purple combines two fundamental colors which are normally never confounded.

3. *Red.* — A vivid, slightly yellowish red. Chosen because it represents the color-group in

which red (orange) and violet (blue) are combined in nearly equal proportions.

Method of Examination and Diagnosis. — Place the Berlin worsteds on the white cloth in which they are wrapped. They should be well mixed, and not spread out too much. Lay a skein of the first test-color in a well-lighted position two or three feet from the group. Inform the person examined:

(1) That he must not speak during the test.

(2) That the skeins are not to be fingered or tossed about. A skein should be touched only after its selection.

(3) That he must endeavor to pick out skeins resembling the test skein, *i. e.*, a little lighter or darker in shade; the resemblance cannot be perfect, as no two shades are exactly alike.

Green Test. — The subject must pick out all the other skeins approximately the same shade.

The color-blind selects some shade of gray.

Purple (*Rose*). — The subject should pick out the skeins of the same color, as before.

(1) He who is color-blind by the first test, and who, upon the second test, selects only purple skeins, is *incompletely purple-blind.*

(2) He who, in the second test, selects with purple only blue and violet, or one of them, is *completely red-blind.*

(3) He who, in the second test, selects with purple only green and gray, or one of them, is *completely green-blind.*

Remark. — The red-blind never selects the colors taken by the green-blind, and *vice versa.* Often the green-blind places a violet or blue skein by the side of the green, but only the brightest shades of these colors. This does not influence the diagnosis.

Red. — This test is applied to those completely color-blind. Continue the test until the person examined has placed beside the specimen all the skeins belonging to this shade, or else, separately, one or more "colors of confusion."

The red-blind chooses (besides the red, green, and brown) shades which to the normal sense seem darker than red. The green-blind selects opposite shades, which seem lighter than red.

Violet Blindness. — Very rare. Recognized by a confusion of purple, red, and orange, in the purple test (see 2). Much care is required to diagnosticate this form.

FERMENTATION

Specific Action

Conversion of Starch to Sugar by Germinating Barley. — To 5 grams crushed, germinating barley add 10 grams potato starch, and 20 c.c. of cold water. Then add gradually 70 c.c. of hot water with constant stirring. Keep the mixture in a temperature of about 60° C. for one hour.

The insoluble starch will be converted to a sweet liquid.[1]

Boil 10 c.c. of Fehling's solution,[2] dilute the syrup with water and add it drop by drop to the boiling Fehling's solution.

[1] Kirchoff: Schweigger's Journal für Chemie und Physik, 1815, xiv, p. 389.

[2] *Fehling's solution.*— In a large watch glass weigh 34.639 gms. pure cupric sulphate (clean crystals). Dissolve the crystals by warming them with about 150 c.c. water in an evaporating dish. Place the solution in a 500-c.c. measuring flask. Wash the remnant from the dish into the flask. Allow the liquid to cool completely. Add water to the mark on the neck of the flask.

Warm about 173 gms. potassium sodium tartrate in a little water until dissolved. Place the solution in a 500-c.c. measuring flask, add 100 c.c. sodium hydroxide, sp. gr. 1.34 (about 31 per cent), and, after the mixture has completely cooled, fill the flask to the mark on the neck.

In use, mix equal volumes of each solution in a dry glass.

One molecule grape sugar reduces five molecules cupric oxide to cuprous oxide; 10 c.c. of Fehling's solution equals 0.05 gm. grape sugar.

Red cuprous oxide or its yellow hydrate will separate.

The germinating barley causes the starch to take up water, thus changing to a reducing sugar. In this instance the agent is a living cell, or some substance or "ferment" secreted by the cell. It is now necessary to inquire whether ferments are separable from living cells.

Conversion of Starch to Sugar by Salivary Diastase (Ptyalin). — To 10 c.c. of starch paste[1] colored blue with iodine (blue iodide of starch) add about 2 c.c. of filtered saliva and keep the mixture at 35–40° C.

The starch paste will liquefy and become sweet. The blue color will become lighter and finally disappear.

Test with Fehling's solution. Reduction will take place.

Saliva hydrolyzes starch to a reducing sugar.

Saliva is secreted by the cells in the salivary gland, placed some distance from the mouth. The saliva itself contains no secreting cells. There are ferments, then, which act at a distance

[1] *Starch paste.* — Rub 1 gm. potato starch in a mortar with 25 c.c. cold water. Pour the mixture into an evaporating dish. Wash the remnant from the mortar and pestle into the dish with 75 c.c. water. Heat the mixture to boiling point with constant stirring. The starch paste will turn blue upon addition of iodine (iodide of starch).

from the cells that produce them. There seems thus an important distinction to be made between organized ferments, those acting apparently within the living cell, and unorganized ferments, like the salivary diastase, which is secreted by a living cell but remains active after leaving the cell. It will be seen that this distinction cannot be maintained.

Extraction of Diastase from Germinating Barley.[1] — Crush freshly germinating barley in a mortar with about half its weight of water. Keep the mass two hours at 35–40° C. Squeeze out the watery extract in a press, or strain by strong pressure through a linen cloth. Add excess of alcohol.

Diastase will be precipitated. It may be purified by dissolving it in water and reprecipitating with alcohol.

Add a little diastase to 10 c.c. starch paste, colored blue with iodine. The starch will be converted to sugar. The blue color will disappear.

It appears, therefore, that ferment action is not dependent on the life of the cell that secretes the ferment.

Specific Action of Ferments. — The question now arises whether the diastase acts only to

[1] Payen and Persoz: Annales de chimie et de physique, 1833, liii, p. 78.

change starch to sugar or whether it causes the decomposition of other substances.

Place a small piece of fibrin in a test-tube and add 2 c.c. filtered saliva. Keep the tube several hours at a temperature of 35–40° C.

The fibrin will not change.

Place 0.5 c.c. neutral olive oil (page 56) and 2 c.c. filtered saliva in a test-tube.

Noteworthy changes will be absent.

From these experiments it is evident that diastase decomposes starches, but does not decompose proteids and fats. Its ferment action is thus far "specific." The belief that each ferment has its own characteristic product will be increased by the study of the following typical ferment actions.

Proteid Digestion by Pepsin

Gastric Digestion of Cooked Beef and Bread. — At 7 A.M. feed cooked beef and bread to a cat which has fasted twelve hours. At 11 A. M. kill the cat, expose the stomach, and apply double ligatures about 1 cm. apart to the duodenum at the pylorus and to the oesophagus at the cardiac orifice. Remove the stomach. Open the stomach very cautiously by drawing a knife along the greater curvature.

"The stomach is very full, and still contains

much meat and bread not wholly softened. The softening is greater in the portal region and in those portions of the food next the mucous membrane than in the middle of the stomach contents. The mucus secreted by the gastric mucous membrane is very abundant and is strongly acid. The stomach contents have a sour odor."[1]

Artificial Gastric Juice.[2] — 1. Strip the mucous membrane from the fourth stomach of a calf. Wash the membrane with cold water until the acid reaction disappears. Dry the mucous membrane in the air. Divide some of the dried membrane into small pieces and add dilute hydrochloric acid.[3]

2. Strip the mucous membrane of the pig or rabbit from the deeper layers of the stomach, cut the mucous membrane into the smallest pieces, wash slightly with water, pour off the water with all possible care, and cover the slightly moist residue with glycerine.[4] Before using, add dilute hydrochloric acid.

[1] Eberle : Physiologie der Verdauung, 1834, p. 100.

[2] Eberle : loc. cit., p. 79.

[3] *Dilute hydrochloric acid.* — Add to 10 c.c. officinal HCl, sp. gr. 1.124 (about 25 per cent HCl), enough water to make 1000 c.c. This solution will contain about 0.281 per cent HCl. (Salkowski's Practicum, 1893, p. 130.)

[4] Von Wittich : Archiv für die gesammte Physiologie, 1869, ii, p. 194.

Digestion with Artificial Gastric Juice. — Prepare three flasks, *A*, *B*, and *C*. In *A* place 100 c.c. artificial gastric juice; in *B*, 100 c.c. 0.2 per cent HCl; and in *C*, a piece of dried gastric membrane and 100 c.c. distilled water. In each of the three flasks place a small piece of cooked meat, and keep the flasks about five hours at 35–40° C.[1] Compare the result with that observed in natural digestion.

The artificial gastric juice will digest the meat as did the natural juice in the stomach, but neither the acid alone, nor the mucous membrane free from acid, will digest. There is a ferment in the mucous membrane, but it will not act except in an acid medium.

Extraction of Pepsin. — Pepsin more or less contaminated with proteid (pepsin may itself be a proteid) may be precipitated from a glycerine extract by alcohol.[2] The pepsin may also be carried down mechanically by an indifferent precipitate as in Brücke's method,[3] in which the mucous membrane, acidulated with phosphoric acid, is allowed to digest until the proteids are mostly converted into soluble peptone. The mixture is then neutralized with lime water. The insoluble calcium phosphate thus formed falls as

[1] Eberle: loc. cit.

[2] Von Wittich: loc. cit., p. 195.

[3] Brücke: Sitzungsberichte der königliche Akademie der Wissenschaften zu Wien, 1862, xliii, p. 601.

a fine powder carrying the pepsin with it. The precipitate is dissolved in very dilute hydrochloric acid, and to this solution is added a solution of cholesterin in alcohol and ether. When the two solutions are mixed, the cholesterin separates as an abundant, fine powder bearing the pepsin with it. The cholesterin is removed with ether, leaving the pepsin.

Ammonium sulphate may also be used as the mechanical precipitant.[1]

Change of Proteid to Peptone by Pepsin. — 1. Place in a test-tube five drops of the glycerine extract of pepsin with 5 c.c. 0.2 per cent hydrochloric acid and a small piece of fibrin.[2] Keep the mixture at 35–40° C.

In a short time the fibrin will be dissolved. Appropriate tests will show that it has been converted to peptone. 2. Repeat the preceding experiment, using commercial pepsin (never very free from proteid).

Splitting of Casein by Rennin.

Rennin Extract. — Allow the mucous membrane of the stomach (preferably the fourth stomach of

[1] Kühne and Chittenden: Zeitschrift für Biologie, 1886, xxii, p. 428.

[2] *Preparation of fibrin.* — With a bundle of smooth rods whip blood as it flows from an artery until the fibrin gathers on the rods. Wash the fibrin in running water until the red corpuscles are removed and the fibrin shows its natural color. Preserve the fibrin in glycerine.

the suckling calf) to stand twenty-four hours in 150–200 c.c. 0.1–0.2 per cent solution of hydrochloric acid. Then neutralize the acid with great care.[1]

Separation of Rennin. — The extract just prepared contains pepsin as well as rennin. The rennin may be separated as follows. The neutralized extract is repeatedly shaken with fresh amounts of magnesium carbonate. The resulting precipitates carry down almost all the pepsin and very little rennin. The filtrate still rapidly coagulates milk, but contains only traces of pepsin. This filtrate is now precipitated with lead acetate, the precipitate is decomposed with very dilute sulphuric acid, and the mixture filtered. To the filtrate, which contains the rennin, is added a solution of stearin soap in water. Thereupon the soap is thrown out of solution and falls, carrying the rennin with it. The soap is then removed by shaking with ether, and the rennin remains.[2]

Precipitation of Casein. — Add 1 c c. of the neutral extract to 25 c.c. fresh milk at 36–38° C. (Normal milk is amphoteric. If the reaction be acid, the acid should be very carefully neutralized.)

In a few minutes the milk will separate into

[1] Hammarsten: Upsala Läkareforenings Förhandlingar, 1872, viii, pp. 63–86. Abstract by author in Maly's Jahresbericht über die Fortschritte der Thierchemie, 1872, ii, pp. 118–125.

[2] Hammarsten: Lehrbuch der physiologischen Chemie, 1895, p. 241.

curd and whey. The curd is casein together with the fat globules carried down as it precipitates. The whey is a dilute saline solution of milk-albumin, milk sugar, etc.

Test the chemical reaction. The mixture is still neutral. Milk may also be curdled by acid, either added artificially or produced in the milk itself by lactic acid fermentation of milk sugar. The absence of an acid reaction in the above experiment excludes precipitation through acid fermentation of milk sugar. Casein prepared free from milk sugar is also precipitated by rennin. Finally, rennin, extracted by the method given above, does not act upon milk sugar, but rapidly precipitates casein.

Analogy suggests that the specific action of the rennin may be the splitting of casein and that the precipitation may be a secondary process. The following experiments determine this matter.

Experiments of Arthus and Pagès.[1] — Prepare two solutions, *A* and *B*.

A.		*B*.	
Milk	100 c.c.	Milk	100 c.c.
Neutral oxalate of potassium 1 %	5 c.c.	Neutral oxalate of potassium 1 %	5 c.c.
Rennin 1 to 250	4 c.c.	Water	4 c.c.

[1] Arthus and Pagès: Archives de physiologie, 1890, p. 334.

(Rennin, 1 to 250, is a pastille of Hansen dissolved in 250 c.c. H_2O.)

Keep both mixtures at 38° C. during forty minutes. 1. Boil 25 c.c. from each solution. Solution *A* coagulates, while solution *B* shows no trace of coagulation. Hence the action of rennin has rendered the casein in *A* coagulable on boiling.

2. To 25 c.c. from each solution add 8 c.c. of a solution of calcium chloride capable of precipitating exactly, in equal volumes, the solution of potassium oxalate. By this addition the calcium oxalate is removed and the calcium chloride remains in slight excess.

A will coagulate; *B* will not. Hence the casein in solution *A* has been so changed by rennin that it is precipitated on the addition of a small quantity of calcium chloride. Solution *A* may also be precipitated by restoring its original content of calcium chloride, i.e. by adding 5 c.c. of the above calcium chloride solution, which will exactly combine with the 5 c.c. of potassium oxalate.

If small quantities of rennin be added to natural milk and equal portions of the milk be tested from time to time by boiling, the amount coagulated will be greater the longer the rennin acts. An amount of calcium chloride too small to produce coagulation in the early stages of

rennin action is sufficient to produce coagulation when added in the later stages.

Evidently, in the clotting of milk by rennin two separate phenomena must be distinguished: (1) the chemical transformation of casein by rennin, (2) the precipitation of the transformed casein by the calcium chloride. (This salt favors also the splitting of the casein.) Rennin may therefore be classed with pepsin and trypsin.

According to Hammarsten the casein is split into phosphorus-free albumose and phosphorus-holding paracasein. Heat is set free. It is the paracasein which precipitates. It is less soluble than casein.

Precipitation of Fibrin by Fibrin Ferment

Buchanan's Experiment. — Press blood clot through a linen cloth. Add the liquid thus obtained to a serous fluid, which does not clot spontaneously, such as ascitic fluid, pleural effusion, hydrocele fluid.

After some hours a firm, translucent clot will form.[1]

Extraction of Fibrin Ferment. *Schmidt's Method.* — Coagulate one part of serum from the blood

[1] Buchanan: London Medical Gazette, 1835, xviii, p. 51; idem, 1845, xxxvi, p. 617. This discovery was first announced in 1831.

of ox, dog, or horse, by adding 15–20 parts strong alcohol. After at least fourteen days, filter, dry the moist residue over sulphuric acid, pulverize the dried substance, stir it with water (twice the volume of the serum originally taken) and after allowing sufficient time for solution, filter. The filtrate contains the fibrin ferment.[1]

Gamgee's Method. — Allow freshly prepared fibrin (obtained by washing a blood clot free from corpuscles) to stand three days in 8.0 per cent solution of sodium chloride. Filter.[2]

The filtrate is rich in fibrin ferment.

Extraction of Fibrinogen. — Receive three volumes of blood directly from an artery into one volume of saturated solution of magnesium sulphate, which will prevent the blood from clotting. Separate the corpuscles from the liquid plasma by the centrifugal machine. Add to the plasma an equal volume of saturated solution of sodium chloride. Flakes of fibrinogen will be precipitated. Filter as quickly as possible, for that purpose dividing the liquid among several funnels each with a folded filter paper. Press the filter papers containing the residue between fresh filter paper, in order to remove the adherent

[1] Schmidt: Archiv für die gesammte Physiologie, 1872, vi, p. 457.

[2] Gamgee: Journal of physiology, 1879, ii, p. 151.

liquid. Tear the filter containing the fibrinogen into small pieces. Dissolve the fibrinogen which sticks to the filter as a tough, elastic mass, in a quantity of 8 per cent sodium chloride solution equal to about one-third the quantity of the magnesium sulphate solution originally taken. Filter off the fragments of paper. Purify by reprecipitation with an equal volume of saturated solution of sodium chloride. Filter. Dry as before, and add a small quantity of water to the finely divided filter to which the precipitate clings. This water will take a small quantity of salt from the precipitate, and in this dilute saline solution the fibrinogen will dissolve.[1]

Precipitation of Fibrinogen by Fibrin Ferment.— Add to the dilute saline solution of fibrinogen a solution containing fibrin ferment.

Fibrin will form.

Ammoniacal Fermentation of Urea by Urease

1. Place 100 c.c. fresh human urine in each of three clean flasks marked *A, B, C.* To *B* and *C* add 1 c.c. of urine that has become ammoniacal upon standing in the atmospheric air. Add also

[1] Hammarsten : Archiv für die gesammte Physiologie, 1879, xix, p. 563. Also idem, 1880, xxii, p. 431. Hammarsten's first publication was in Nova acta regia societas scientiarum Upsaliensis, 1878, (3), ix.

to *C* 2 per cent of a saturated solution of carbolic acid in water. Let *B* and *C* stand in a warm place sixteen days.

2. Withdraw 5 c.c. from flask *A*. Note whether the urine is clear or turbid, and whether it effervesces on the addition of a dilute acid. Withdraw 2 c.c. from flask *A* and determine its percentage of urea by the hypobromite method.

Centrifugalize a portion of the remaining contents of flask *A*. With a microscope examine the sediment for crystals of ammonio-magnesium phosphate and for micro-organisms, especially the micrococcus ureæ, which occurs in long curved chains of round cells about 1.5 μ in diameter.

3. After sixteen days repeat these observations on the urine in flasks *B* and *C*. Record the results obtained from all three flasks in the table on page 52.

The table shows that the hydrolysis of urea into ammonium carbonate still takes place in urine containing enough carbolic acid to destroy the micro-organisms long known to be the cause of the ammoniacal fermentation.[1] It is therefore probably due to a ferment, which escapes from the cells after their death.

[1] Hoppe-Seyler: Medicinisch-chemische Untersuchungen, Berlin, 1866, p. 570.

Flask.	Content of Carbolic Acid.	Clear or Turbid.	Reaction to Acids.	Per cent of Urea.	Crystals of Ammonio-Magnesium Phosphate.	Micro-Organisms.
A NORMAL						
B SEPTIC						
C ASEPTIC						

Prior to 1860 ammoniacal decomposition of urine was vaguely classed as a fermentation. In that year Müller[1] suggested that it might be due to a body like beer-yeast. In 1862 Pasteur[2] discovered such a yeast, which he called *Torula ureæ*. Cohn first classed it with the micrococci. It is aerobic. Miguel finds seven species of bacilli, nine micrococci, and one sarcina, that decompose urea. These obtain their nitrogen ordinarily from proteids, but in the absence of proteids may utilize urea.

Extraction of Urease. — To 10 c.c. of urine undergoing an active ammoniacal fermentation, add 50 c.c. of strong alcohol, and allow the mixture to stand in a well-corked flask. After five days place the precipitate upon a very small filter and wash it with 50 c.c. of fresh alcohol. (Preserve both filtrates for recovery of the alcohol by redistillation.)

1. Add a very small quantity of this precipitate to a neutral 2 per cent solution of urea. Test the reaction. Place the mixture in a water bath at 38° C.

After a few minutes again test the reaction.

It will be strongly alkaline.

[1] Müller: Journal für praktische Chemie, 1860, lxxxi, p. 467.

[2] Pasteur: Comptes rendus de l'académie des sciences, Paris, 1860, l, p. 869. See also Van Tieghem, idem, 1864, p. 210.

After a short time the odor of ammonia will be perceptible. The alcoholic precipitate contains a ferment capable of quickly hydrating urea.

"The alcoholic precipitate from the unfiltered urine consists chiefly of various salts together with the cells of the Torula, hence when treated with water some of the salts are dissolved and pass with the ferment through the filter. If this first aqueous extract be again precipitated with alcohol, a portion of the salts will be again removed, and if this second precipitate be several times redissolved in water and reprecipitated with alcohol, the body with the ferment properties may be ultimately separated — as an amorphous white powder soluble to a clear solution in distilled water and not characterized by any special chemical reactions."

The ferment is not secreted by the cells into the surrounding liquid, but is retained within the cell bodies, for the living cells may be filtered off, and the filtrate will not hydrate the urea.[1]

Splitting and Synthesis of Fats

Chemistry of Fats and Soaps. — When olive oil is saponified, glycerine appears (Scheele, 1779).

[1] Lea : Journal of Physiology, 1885, vi, p. 138. See also Musculus: Comptes rendus de l'académie des sciences, Paris, 1874, lxxviii, p. 132; idem, 1876, lxxxii, p. 333; Archiv für die gesammte Physiologie, 1876, xii, p. 214.

It is related to the alcohols (Chevreul, 1813), being a compound ether or ester, a combination of an alcohol with an acid. Commercially glycerine is prepared by exposing neutral fats, such as stearin, to superheated steam, whereby the neutral fat is split into glycerine and fatty acid.

$$\begin{array}{l} CH_2O\cdot CO(CH_2)_{16}\cdot CH_3 \\ CHO\cdot CO(CH_2)_{16}\cdot CH_3 \\ CH_2O\cdot CO(CH_2)_{16}\cdot CH_3 \\ \text{STEARIN} \end{array} + \begin{array}{l} H\cdot OH \\ H\cdot OH \\ H\cdot OH \\ \text{WATER} \end{array} =$$

$$\begin{array}{l} CH_2\cdot OH \\ CH\cdot OH \\ CH_2\cdot OH \\ \text{GLYCERINE} \end{array} + \begin{array}{l} CO\cdot OH(CH_2)_{16}\cdot CH \\ CO\cdot OH(CH_2)_{16}\cdot CH_3 \\ CO\cdot OH(CH_2)_{16}\cdot CH_3 \\ \text{STEARIC ACID} \end{array}$$

If an alkali be present, it will combine with the fatty acid to form a soap.

$$\begin{array}{l} CO\cdot OH(CH_2)_{16}\cdot CH_3 \\ CO\cdot OH(CH_2)_{16}\cdot CH_3 \\ CO\cdot OH(CH_2)_{16}\cdot CH_3 \\ \text{STEARIC ACID} \end{array} + \begin{array}{l} Na\cdot OH \\ Na\cdot OH \\ Na\cdot OH \\ \text{SODIUM HYDROXIDE} \end{array} =$$

$$\begin{array}{l} CO\cdot ONa(CH_2)_{16}\cdot CH_3 \\ CO\cdot ONa(CH_2)_{16}\cdot CH_3 \\ CO\cdot ONa(CH_2)_{16}\cdot CH_3 \\ \text{SODIUM STEARATE} \end{array} + \begin{array}{l} H\cdot OH \\ H\cdot OH \\ H\cdot OH \\ \text{WATER} \end{array}$$

Splitting of Fats by the Pancreatic Juice. *Bernard's Experiment.* — Place 2 c.c. neutral olive

oil in a test-tube and add a small quantity of pancreatic juice (or a piece of fresh pancreas or extract of pancreas). Test the reaction of the mixture. It is alkaline. Note that a white, creamy liquid forms almost immediately. This "emulsion" is composed of a multitude of small fat globules.

Test the reaction again. It gradually becomes acid.

It is evident that under the influence of the pancreatic juice the fatty matter is not simply finely divided and emulsified, but that it has also been modified chemically.[1]

In order to study the splitting of neutral fats by lipase, a ferment found in the pancreatic juice, it is necessary (1) to prepare a perfectly neutral fat, and (2) to recognize the fatty acid as soon as it is set free.

Preparation of Neutral Fat. — Shake commercial olive oil (which always contains fatty acid) for two hours at 95° C. in a separating funnel with a saturated solution of barium hydroxide. Allow the mixture to stand until the oil separates from the hydroxide. Remove the hydroxide. Filter the oil.

The Emulsion Test for Fatty Acid. *Brücke's*

[1] Bernard : Comptes rendus de l'académie des sciences, Paris, 1849, xxviii, p. 250.

Experiment. — 1. Shake 1 c.c. neutral olive oil in a test-tube with 5 c.c. 0.25 per cent sodium carbonate solution.

The oil will be broken up into large globules which will speedily reunite, leaving the liquid clear.

2. Shake 1 c.c. rancid olive oil (containing about 5.5 per cent fatty acid) with 5 c.c. 0.25 per cent sodium carbonate solution.

The mixture becomes instantly milky. The oil is divided into globules of microscopic size. The emulsion is permanent.

3. Shake 1 c.c. neutral olive oil with 5 c.c. water.

The water and oil will not mix.

4. Shake 1 c.c. neutral oil with water containing soap.

The oil will be emulsified. It is probable therefore that soap contributes to the emulsion, perhaps by coating the fine particles of oil with a membrane that prevents their reunion.[1]

Gad's Experiment. — 1. Fill a watch glass about 5 cm. in diameter with 0.25 per cent solution of sodium carbonate. With a glass rod carefully place a large drop of rancid olive oil (containing 5.5 per cent fatty acid) upon the surface of the soda solution.

[1] Brücke: Sitzungsberichte der kaiserlichen Akademie der Wissenschaften zu Wien, 1870, lxi, pp. 613–614.

The drop will come to rest, and for a moment both the drop and the surrounding liquid remain clear. Very soon, however, the oil is covered with a white layer, and through the soda solution spreads a white cloud which becomes denser and denser until the oil drop, steadily diminishing in size, floats in a milky white liquid.

2. Repeat the experiment, observing the oil drop under a low power of the microscope.

Note the extraordinary motion in the neighborhood of the oil drop, and how the particles of oil are thrown out in strong eddies.

3. Examine the completed emulsion under a higher power of the microscope.

There appear exceedingly small fat drops of very uniform size. The milky fluid is the finest and most uniform emulsion.[1]

Rachford's Experiment. — "Arrange a series of watch glasses containing 0.25 per cent solution sodium carbonate. Place in a test-tube 2 c.c. neutral olive oil and 1 c.c. pancreatic juice (or extract). Shake the tube and allow the juice and oil to separate, then pipette a drop of oil from the surface and place it on the soda solution in watch glass 1. Again shake the tube and allow the oil and juice to separate, then pipette as before, placing a drop of oil in watch

[1] Gad: Archiv für Physiologie, 1878, p. 183.

glass 2. Again shake and pipette as before, and repeat this process every three or four minutes until the experiment is completed. The beginning of the experiment and the time of each pipetting must be carefully noted. If the pipettings are three minutes apart, then the first drop of oil will have been exposed three minutes to the action of the pancreatic juice, the second drop six minutes, the third nine minutes, and so on.[1]

The gradual increase in fatty acid will be shown by the gradual increase in the amount of the spontaneous emulsion.[2]

It has just been shown that lipase will hydrolyze neutral fats into fatty acid and glycerine. We must now enquire whether this ferment can effect the synthesis of fats, in other words whether its action is reversible. For this pur-

[1] Rachford: Journal of physiology, 1891, xii, p. 81. Rachford used $\frac{1}{3}$ c.c. fresh pancreatic juice obtained by placing a glass tube in the pancreatic duct of the rabbit (see page 80).

[2] "There is a possible error in this method which had better be spoken of here. It would seem that the alkali of the pancreatic juice would combine with the fatty acids forming soap, and in this way the oil would soon be emulsified in the juice itself and not separate after shaking. This would indeed be a serious drawback if it actually occurred, but in truth it does not occur until late in the experiment after we have obtained the information we have sought by the spontaneous emulsion method." (Rachford, loc. cit., p. 82).

pose an extract of lipase may be used, first, to split a neutral fat (or glycerol ester) into its constituent fatty acid and alcohol (glycerine is a trihydric alcohol), and second, to form a neutral fat from fatty acid and alcohol.

Extraction of Lipase. *From Pancreas.* — Remove the pancreas of the pig within thirty minutes after the death of the animal. Dissect off as much of the fat as possible. Reduce the pancreas to a fine pulp in a mortar with coarse well-washed white sand. Extract the lipase with a little water or glycerine.

From Liver. — Remove the liver of the pig within thirty minutes of the death of the animal. Reduce 50 gms. to a fine pulp in a mortar with about 200 c.c. water. Filter. Dilute the watery extract to 500 c.c.

Hydrolysis of Ethyl Butyrate by Lipase. — Place in each of two test-tubes, *A* and *B*, 4 c.c. water, 0.1 c.c. toluene,[1] and 0.26 c.c. ethyl butyrate.[2] Cork the tubes tightly. Place them in the water bath for five minutes, to bring them to the temperature of the bath, 40° C. Add 1 c.c. of

[1] Toluene is an antiseptic, which prevents the splitting of the neutral fat by bacteria.

[2] Ethyl butyrate hydrolyzes more rapidly than butter fat. It has the further advantage that the amount split by the temperatures employed during the time of the experiment is too small to be measurable.

the aqueous extract of lipase to each. Boil tube *B*. Place both tubes at 40° C. for fifteen minutes. Remove them from the bath and plunge them into ice-water (to check further ferment action). Titrate with $\frac{n}{20}$ KOH, using neutral litmus as the indicator.[1] The initial acidity of the

[1] A *normal* solution contains in each litre one equivalent weight of the active substance, *i. e.* that mass of the active substance which is equivalent to the atomic weight of a univalent element in the reaction for which the normal solution is to be employed. Equal volumes of different normal solutions are equivalent to each other. Thus, 1 c.c. normal alkali solution requires for neutralization exactly 1 c.c. normal acid, no matter what acid is employed to make the normal solution.

Preparation of Normal Potassium Solution. — The content of KOH in 1 litre is 56.16 grams. Dissolve 60 gms. purest commercial KOH (which always contains considerable water) in a graduated cylinder in about 950 c.c. water. Determine the true content of KOH by titration with a normal oxalic acid solution (prepared by dissolving its equivalent weight 63 gms. in 1 litre water) as follows. Thoroughly stir the potassium hydroxide solution, fill a burette with a portion of the well-mixed solution. Place 10 c.c. normal oxalic acid solution in a beaker and add a few drops of solution of rosolic acid as indicator. Add the alkali from the burette cautiously until the end point of the reaction is reached, *i. e.* until the indicator gives a red color which does not quickly disappear. As 10 c.c. of acid solution should exactly neutralize 10 c.c. of alkali solution, provided both were normal, it follows that the quantity of KOH solution necessary to neutralize is to 10 c.c. as the total quantity of the original KOH solution is to x. x will be the number of cubic centimetres to which the KOH solution must be diluted in order to make it normal. A portion of the normal solution should then be diluted 1:20, and preserved in an air-tight

enzyme solution, usually 0.1 to 0.2 c.c. $\frac{n}{20}$ KOH, should be deducted from the cubic centimetres KOH required to neutralize the fatty acid formed.[1]

Fatty acid will appear in tube *A*, but not in tube *B*, in which the enzyme was destroyed by boiling.

Synthesis of Neutral Fat by Lipase. — 1. Place 5 c.c. $\frac{n}{100}$ butyric acid, 2 c.c. 13 per cent alcohol, 1 c.c. diluted glycerine extract of pig's pancreas (or aqueous extract of liver) in each of two test-tubes, *A* and *B*. Boil the contents of test-tube *B*. Seal both tubes. Keep them thirty-six hours at 48.5° C.

On opening the tubes, *A* will give a distinct odor of ethyl butyrate; none will be found in *B*, in which the ferment was destroyed by boiling.[2]

2. Place 5 gms. glycerine, 2 gms. isobutyric acid, 125 gms. water, 1 c.c. neutralized blood serum (or aqueous extract of pig's liver) in each of two

flask. (Compare Müller and Kiliani: Kurzes Lehrbuch der analytischen Chemie, 1900, p. 31 and p. 83).

At 30° (summer temperature) 0.26 c.c. ethyl butyrate weighs 0.2300 gram. This quantity, if completely hydrolyzed, would require 39.7 c.c. $\frac{n}{20}$ KOH.

[1] Kastle and Loevenhart: American chemical journal, 1900, xxiv, pp. 491–525. Also Loevenhart: American journal of physiology, 1902, vi, pp. 331–350.

[2] Kastle and Loevenhart: loc. cit., p. 518.

test-tubes, *A* and *B*. Boil the contents of tube *B*. Place both at 37° C. At intervals of half an hour titrate a portion from each tube with $\frac{n}{20}$ KOH solution. The acidity will diminish in both, but much more rapidly in the tube containing the active ferment.

The acidity is diminished by the combination of the fatty acid with the glycerine to form a neutral fat.[1]

Fats are hydrolyzed to some extent in the stomach,[2] but stomach lipase is active only in neutral solutions. It is inhibited or destroyed by 0.3 per cent hydrochloric acid. Other ethereal salts besides the fats are hydrolyzed in the intestine, *e. g.* salol.[3]

The rate of change by lipase increases with the amount of the enzyme present.[4]

Reversible action is seen in ferments other than lipase, as in the following experiments.

Splitting of Hippuric Acid by Histozyme. — A pig's kidney was perfused four hours with one litre defibrinated pig's blood to which 0.8 gram hippuric acid

[1] Hanriot: Comptes rendus de la société de biologie, 1901, p. 70.

[2] Marcet: Proceedings Royal Society, London, 1858, ix, p. 306. Ogata: Archiv für Physiologie, 1881, p. 515. Cash: Archiv für Physiologie, 1880, p. 323.

[3] Baas: Zeitschrift fur physiologische Chemie, 1890, xiv, p. 416.

[4] Kastle and Loevenhart: loc. cit., p. 511.

(sodium salt) had been added. The blood passed through the kidney 9–10 times.

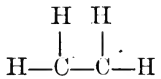
Upon analysis, there appeared 0.037 gram benzoic acid, produced from 0.1276 gram hippuric acid.

Synthesis of Hippuric Acid by Histozyme. — A pig's kidney was perfused three hours with one litre defibrinated pig's blood containing a neutral solution of 0.5 gram benzoic acid and 0.6 gram glycocoll. The blood passed ten times through the kidney.

Found: 94 mgm. hippuric acid.[1]

These actions depend upon a ferment, histozyme, extracted by Schmiedeberg.

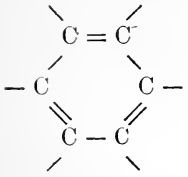
Some hypothetical considerations will be of value here. Compounds of carbon may be divided into those in which the carbon atoms are arranged in an open chain, for example ethane, C_2H_6,



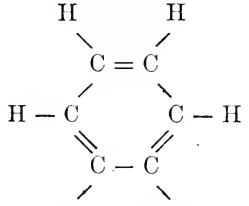
ETHANE

and those in which the chain is closed to form a "carbon ring," for example, benzene, C_6H_6, which consists of six carbon atoms, in a closed, ring-shaped chain, the "benzene nucleus," with a hydrogen atom joined to each carbon atom by its fourth affinity (Kekulé, 1865).

[1] Schmiedeberg: Archiv für experimentelle Pathologie und Pharmakologie, 1881, xiv, pp. 382–383.



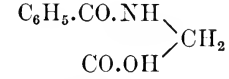
BENZENE NUCLEUS OR RING



BENZENE

The benzene ring is not easily opened, but derivatives of benzene may be readily obtained by replacing hydrogen atoms. Thus, in aniline or amido-benzene, $C_6H_5.NH_2$, one hydrogen atom is replaced by amide radical; in carbolic acid, or phenol, $C_6H_5.OH$, by hydroxyl; in toluene or methyl benzene, $C_6H_5.CH_3$, by the radical CH_3. The carbon atom in methyl benzene is not a part of the benzene ring, but is chained to the side of the ring. The hydrogen atoms in the side-chain differ in their affinities from those attached to the ring; the hydrogen in the ring may be replaced by groups (*e. g.* NO_2) which will not readily replace the hydrogen of the side-chain. This is a matter of special interest in relation to the specific action of poisons, ferments, etc. By substituting hydroxyl for the hydrogen of the side-chain, benzyl alcohol, $C_6H_5.CH_2.OH$, is formed. By introducing carboxyl, benzoic acid, $C_6H_5.CO.OH$, is obtained. It has been shown above that benzoic acid and glycocoll are united in the kidney to form hippuric acid. Glycocoll is amido-acetic acid, $CH_2(NH_2).CO.OH$. It

unites with benzoic acid by replacing the hydroxyl in the side-chain, thus forming



HIPPURIC ACID

Cinnamic acid, toluene, and other aromatic substances are similarly excreted as hippuric acid when taken internally.

The reversible action of the kidney ferment is important in hastening the establishment of the equilibrium between benzoic acid and glycocoll. If these two bodies pass through the kidney, a certain amount of hippuric acid is formed; if hippuric acid itself passes through the kidney, a certain quantity is hydrolyzed.

Relation of Reversible Action to Absorption of Fat.— "Pancreatic juice is capable of hydrolyzing all the fat of a fatty meal in the period of pancreatic digestion. In the living intestine the hydrolysis should be complete, inasmuch as the removal of the products of the hydrolysis by absorption prevents the establishment of equilibrium. On the other hand, the products of the hydrolysis in their transition through the epithelial cells come in contact with a lipolytic enzyme, the presence of which in these cells has been demonstrated in the above.

"The lipase now finds itself in contact with only fatty acid and glycerine, and hence in acting catalytically to bring about the chemical equilibrium, it effects

the synthesis of a fat. This would offer a satisfactory explanation of the presence of fat granules in these cells. As the fatty acid and glycerine diffuse out of the cells through the basement membrane, the fat in these cells would speedily disappear were it not that these substances were constantly being absorbed from the lumen of the intestine. When absorption ceases, however, the fat present is at once hydrolyzed by the lipase present. This hydrolysis is in all probability complete for the reason that the products of the hydrolysis, *viz.*, glycerine and fatty acid, are being constantly removed by diffusion. According to this view, therefore, no fat ever enters or leaves the epithelial cells as such, but as fatty acid and glycerine.

"These two substances then enter the central lacteal, where equilibrium is again established and there is a large production of fat."[1]

IMMUNITY

Ehrlich's Ricin Experiments.[2] — Powder Albert biscuits weighing 6.75 grams. Add to each cake

[1] Kastle and Loevenhart: loc cit., p. 522.

[2] Ehrlich: Deutsche medicinische Wochenschrift, 1891, xvii, pp. 976–979.

Ricin is a toxalbumin extracted from the seeds of the castor oil plant. It is poisonous in the slightest traces. Weight for weight it is a billion times more poisonous than corrosive sublimate. Intravenous injection of 0.03 milligram (0.00003 gram) per kilo of body weight is fatal. One gram commercial ricin would kill one and one-half million guinea-pigs. The effect is about one hundred times less when taken by the mouth, yet

3.2–3.5 c.c. of water containing ricin. The beginning content of ricin should be 0.02 gm. ricin for each cake; 0.035 gm. is fatal in the course of five or six days. Mix the biscuit powder and ricin solution to a stiff dough, roll the dough into rods, divide them into equal lengths, and dry the portions quickly on a wire sieve. Determine the effect on white mice of successively increasing doses, as follows:

DAY	DOSE	DAY	DOSE
1	0.002 gm.	9	0.02
2	. . .	10	0.03
3	0.006	11	0.04
4	0.008	12	0.05
5	. . .	13	0.06
6	0.01	14	. . .
7	0.0125	15	0.08
8	0.015	16	0.01

On the 17th day inject subcutaneously a fresh mouse with the fatal dose — 1 c.c. of a $\frac{1}{200000}$ solution per 20 gm. of mouse. At the same time

even thus 0.18 gram will kill a full-grown man. The cause of death is agglutination of red blood corpuscles, and hence multiple thrombosis, especially of the abdominal vessels. Clinically, violent diarrhœa and progressive exhaustion are observed. The toxicity is greatly dependent on species. Guinea-pigs are far more susceptible than white mice. With white mice the fatal subcutaneous injection is 1 c.c. of a solution containing $\frac{1}{200000}$ ricin per 20 grams of body weight.

inject the immunized mice with a dose one hundred times as great.[1]

Observe the non-immune and the immune mice for several days and note the results.

Ehrlich continued the above experiment until the immunized mouse received daily 0.5 gm. of the ricin by the mouth. Such animals bore safely subcutaneous injections of $\frac{1}{500}$ and even more. The immunity also appeared in that solutions of 0.5–1.0 per cent applied to the eyes of non-immune mice caused violent panophthalmitis, while immune mice bore easily the application of 10 per cent solutions.

This absolute *local* immunity was fully established when the *general* immunity had attained only a middle grade. Normally the subcutaneous injection of $\frac{1}{40000}$ ricin solution causes severe local inflammation, but thoroughly immunized animals bear $\frac{1}{1000}$. Quantitative experiments show that the resistance to the poison is not increased during the first four days, and the increase is doubtful on the fifth day, but on the sixth day a relatively high (for example thirteenfold) general immunity is suddenly established. The sudden fall toward normal temperature observed in diseases with a "crisis," such as pneumonia, may depend on the "critical" establishment of immunity.

Immunity is not increased by continued administration of the same dose, day by day. An equilibrium appears to be established.

[1] The mice in these experiments must be carefully protected against cold and wetting.

The immunity once established endures a considerable time; six months and possibly much longer.

Ricin Antitoxine. — Defibrinate the blood of the immunized mice. Divide it into two portions. 1. To one portion add ricin solution in such a ratio that the mixture shall contain $\frac{1}{100000}$, *i. e.* twice the fatal amount.

Inject a fresh mouse subcutaneously with 1 c.c. of this mixture per 20 grams of weight.

The poison will be borne. It has been neutralized by the serum of the immune animal. This result accords with the discovery of Behring and Kitasato that immunity in diphtheria and tetanus depends on the power of the serum to neutralize the poison.

2. Divide the second portion of the antitoxine blood among six small test-tubes. To the first add a few drops $\frac{1}{100000}$ ricin solution. To the others add amounts increasing in a definite ratio.

At first there will be no effect (immunity). As the amount of ricin added is increased, a point will be reached at which agglutination of red corpusles will be produced. This is the neutralization point.

Evidently, there is a definite quantitative chemical relation between the toxine and the antitoxine.

Theory of Immunity.[1] — Jenner discovered the protective action of vaccinia against small-pox. The small-pox virus when passed through a susceptible animal becomes attenuated. This weakened poison introduced into the circulation in man protects the individual for long periods against the original disease — it establishes an artificial immunity against small-pox. Schwann found that fermentation and putrefaction arose through the agency of micro-organisms coming from without. Pasteur and Koch demonstrated that the inoculation of animals with pure cultures of certain bacteria produced specific infectious diseases, and that these cultures could be modified at will, either by passing through the animal body, as in Jenner's method, or in artificial culture media. Pasteur produced artificial immunity by using attenuated virus. Behring discovered that the blood-serum of animals immunized against diphtheria contained a substance which would protect other animals against the toxine of diphtheria. So also with tetanus. Ehrlich introduced the quantitative study of toxines and antitoxines by means of test-tube experiments, thereby eliminating the uncertain factor of the animal body. Thus it was shown in experiments on tetanus toxine that the action of antitoxines is accelerated by heat, retarded by cold, dependent on concentration — in short, that it is a chemical action. In the above experiments on ricin, it is shown that the relation

[1] Ehrlich: Croonian Lecture, Proceedings of the Royal Society, London, 1901, lxvi, pp. 424–448.

between toxine and antitoxine is quantitative. These results, obtained by test-tube experiments, have been confirmed by observations on living animals. Thus it was established that a fixed quantity of toxine is neutralized by a fixed quantity of its specific antitoxine.

Chemical substances affect only those tissues with which they are able to come into chemical contact. They must first reach the tissue. This general law is illustrated by the experiments of Dönitz with tetanus toxine.[1] When the toxine is injected directly into the circulation and immediately followed by a chemically equivalent amount of antitoxine, the animal is not poisoned; all the toxine circulating in the blood is neutralized. When the same neutralizing dose is injected eight minutes after the toxine, death occurs from tetanus exactly as if no antitoxine had been used. In these eight minutes a lethal quantity of toxine must have left the blood and entered the tissues. This toxine which has entered the tissues may still for a time be withdrawn by injection of the specific antitoxine in quantities much greater than the simple neutralizing dose. The longer the delay, the larger the saving dose. But after a fixed interval, or "period of incubation," no amount of antitoxine, however large, will prevent tetanus. There must, therefore, be present in the brain or cord (the organ principally affected by tetanus toxine) certain atom groups which, like the antitoxine, have a chemical affinity for the toxine. At the close of the period of incuba-

[1] Dönitz: Klinisches Jahrbuch, 1900, vii.

tion the chemical union between these atom groups and the toxine is complete and the antitoxine is shut out. Wassermann [1] found that when tetanus toxine was mixed with fresh brain or cord substance from the guinea-pig, the toxine united chemically with the nerve centres so that neither the surrounding liquid nor the mixture itself was poisonous when injected into an animal.

The stable benzene ring and the less stable side-chains of the benzene derivatives [2] suggested to Ehrlich that living cells also consist of a stable centre and less stable side-chains. The side-chains enable the cell to form chemical combinations with food stuffs and other bodies that possess atom groups having a chemical affinity with the atom groups in the side-chains. It is in this way that the toxine is bound to the cell. Experiments have shown that the binding atoms in the toxine molecule are not the poison atoms. If for a portion of fresh toxine there be determined quantitatively (1) the killing power and (2) the amount of antitoxine required to neutralize the toxine, and if the remainder of the toxine be then allowed to stand for a time, it will be found, on again determining the toxic power and the combining power, that the toxic power has diminished, while the combining power remains almost the same. Hence, two separate and independent groups exist. Ehrlich terms the combining atoms the haptophore group, while the poison atoms are the toxophore

[1] Wassermann: Berliner klinische Wochenschrift, 1898.

[2] See page 65.

group. The haptophore atom group (ἅπτω, I cling to) unites with the antitoxine, if there be any present, or with any other atom group for which it has chemical affinity. If this latter atom group be in the side-chain of a living cell, its union with the haptophore atoms of the toxine will necessarily bring the poison atoms of the toxine into intimate chemical relationship with the central atoms of the cell. Poisoning will then take place. If the cells of vital organs have no atom groups with chemical affinity for the haptophore group of a toxine, no union between cell-atom group and haptophore takes place, the toxophore is not brought into intimate contact with the cell, and poisoning does not occur. The animal is naturally immune to this particular toxine. Thus a toxine in sausages is excessively poisonous to man, the monkey, and the rabbit, while even large amounts are not injurious to the dog.

The haptophore group of the toxine acts immediately after injection into the organism, while in most or all toxines the toxophore group becomes active only after a longer or shorter incubation period. During this period the animal may often be saved by placing it in conditions in which the toxophores cannot act. Thus frogs kept at less than 20° C. are not poisoned by large doses of tetanus toxine, though much smaller doses are fatal at a higher temperature (Morgenroth).

The toxophile atom group of the cell was not predestined to unite with a remotely possible toxine, — it has a normal function, probably that of attaching food to the cell. When it enters into its firm and

enduring union with the haptophore group of a toxine, this normal function is lost. Such a loss acts as a physiological stimulus.[1] New side-chains are produced by the cell, only to unite with fresh toxine. The production and the loss of side-chains continue until all the toxine in the blood is neutralized. By this time the cell has become habituated to a more than normal production of these special atom groups. The excess is cast off like a secretion and circulates in the blood. These free side-chains, possessing a special affinity for one specific toxine, constitute the antitoxine of that toxine.

Their continued production after the neutralization of all the toxine protects the animal against fresh toxine, *i. e.* establishes continued immunity.

It has already been stated that by special means the toxophore group of a toxine may be weakened or destroyed while its haptophore group is unchanged. Such altered and non-poisonous toxines are termed toxoids. As their affinity for the side-chains of the cells remains unaltered, the toxoids by continuing to unite with the side-chains of the cells may stimulate the production of such side-chains in excess, or, in other words, may assist in making antitoxine and thus establishing immunity.

[1] Weigert: Deutsche medicinische Wochenschrift, 1896.

Haemolytic and Bacteriolytic Ferments

Bordet's Experiments.[1] — Inject into the peritoneum of a guinea-pig 10 c.c. defibrinated rabbit blood on five successive days. After two more days bleed the guinea-pig and obtain the serum, by allowing the blood to stand in test-tubes in a cool place until the shrinking clot has pressed out the serum.

1. Mix a drop of serum from a fresh guinea-pig (one not injected with rabbit blood) with a drop of defibrinated rabbit blood and examine under the microscope. The corpuscles show a very slight agglutination, but are otherwise uninjured. The normal serum of the guinea-pig is almost inactive upon rabbit blood.

2. A. Mix a drop of the serum from the injected guinea-pig with a drop of defibrinated rabbit blood and examine under the microscope. The corpuscles are strongly agglutinated.[2]

B. Mix 0.5 c.c. of the serum with 1.5 c.c. defibrinated rabbit blood.

[1] Bordet: Annales de l'Institut Pasteur, 1898, xii, pp. 692–694.

[2] Agglutinated blood looks granular, especially on gentle shaking; the massed corpuscles sink rapidly; they will not pass through filter paper. Agglutination of blood corpuscles is similar to the clumping of the typhoid bacillus in the serum of a typhoid-fever patient.

The corpuscles are agglutinated and their haemoglobin is set free. The mixture becomes red, clear and limpid in two or three minutes. With the microscope nothing can be found but the stroma of the corpuscles, more or less deformed, very transparent and scarcely visible.

The continued presence of blood corpuscles of the rabbit in the blood of the guinea-pig has developed in the latter the power to agglutinate the corpuscles and to set free their haemoglobin. It is thus that the guinea-pig protects itself; it acquires immunity.

3. Heat 1 c.c. of serum to 55° C. for half an hour. Add 0.5 c.c. of this to 1.5 c.c. defibrinated rabbit blood as in Experiment 3.

The serum which was heated to 55° C. no longer destroys the corpuscles, but still strongly agglutinates them.[1]

Evidently the agglutination of the corpuscles and the setting free of the haemoglobin (termed "laking") are effected by different substances. The agglutinating body resists a temperature that destroys the blood-laking body.

4. To the mixture used in the preceding experiment, add 2 c.c. of fresh serum from a normal

[1] A very slow destruction of the red corpuscles may be observed. This, however, is due to the fresh serum in the 1.5 c.c. defibrinated rabbit blood, as will be evident from Experiment 4.

guinea-pig (one that has not been injected with rabbit blood).

In a few minutes the mixture becomes limpid and red. The laking power is restored.

Obviously, with the fresh serum was added the unstable body destructive to red corpuscles. Ehrlich and Morgenroth have shown that at low temperatures the stable body unites with the red corpuscles while the unstable body remains in the serum; in this case the haemoglobin is not set free. At higher temperatures the haemoglobin separates and the unstable body is found to have left the serum. It has joined the stable body in the sediment.

Following the side-chain theory already mentioned, Ehrlich and Morgenroth assume that the stable substance has two combining powers; on the one hand it unites with the red corpuscles, on the other with the unstable substance, thus bringing it to the cell which it may then destroy.

Immunity against toxines and foreign red corpuscles are only two of the protective actions of the blood. The injection of cells of the most varied kinds is followed by the production of specific protective bodies;[1] thus, the injection of

[1] Metchnikoff: Annales de l'Institut Pasteur, 1900, xiv, p. 369.

bacteria causes the formation of bacteriolysines, which destroy the injurious organism.

Many haemolysines and agglutines are found in plants; others, for example, the tetanus bacillus, are bacterial; still others, such as snake venom, are animal secretions.

Oxidizing Ferments

Schönbein's Experiment.[1] — 1. To ten grams hydrogen peroxide add tincture of guiac (freshly prepared by dissolving guiac resin in alcohol) drop by drop until the liquid is milky. Now add from eight to ten drops of a somewhat concentrated extract of malt, prepared in the cold.

The guiac will be oxidized and will turn blue.

2. Repeat the experiment, adding in place of the malt extract from eight to ten drops of blood.

The guiac will be oxidized, as before.

Further Oxidations by Animal Tissues.[2] — 1. Soak strips of bibulous paper in a diluted solution made as follows:

a-naphthol	1 mol.
sodium carbonate	3 "
para-phenylenediamine . .	1 "

[1] Schönbein: Zeitschrift für Biologie, 1868, iv, p. 367.

[2] Spitzer: Archiv für die gesammte Physiologie, 1895, lx, pp. 322–323.

This solution, left in the atmosphere, oxidizes slowly to indophenol (violet color).

Place a drop of a known oxidizer, *e.g.* ferricyanide of potash or potassium chromate, on the saturated paper.

The color will change at once, in consequence of immediate oxidation.

(1) $\underset{\text{PARA-PHENYLENEDIAMINE}}{C_6H_4(NH_2)_2} + \underset{\text{A-NAPHTHOL}}{C_{10}H_7OH} + O = NH\!<\!\begin{matrix}C_6H_4NH_2\\C_{10}H_6OH\end{matrix} + H_2O$

(2) $NH\!<\!\begin{matrix}C_6H_4NH_2\\C_{10}H_6OH\end{matrix} + O = N\!<\!\begin{matrix}C_6H_4NH_2\\\underbrace{C_{10}H_6O}_{\text{INDOPHENOL}}\end{matrix} + H_2O$

Each of the combining molecules has been acted upon by a different oxygen atom; hence the oxygen molecule must have been split.

2. Rub the test paper with finely divided tissue from the liver or any other organ.

Oxidation will occur.

A drop of blood placed on the test paper is soon surrounded by a characteristically colored ring.

Extraction of Nucleo-Proteid from Liver.[1] — Perfuse a fresh liver (dog) with tap water until the washings are no longer colored by haemo-

[1] Spitzer: Archiv für die gesammte Physiologie, 1897, lxvii, p. 616.

globin. Grind the liver to a pulp and press through several thicknesses of gauze. Add five volumes of distilled water. Allow the mixture to stand twenty-four hours at low temperatures. Remove the opalescent watery extract with a pipette and filter through linen. Demonstrate with the microscope that liver cells are absent from the liquid. Add $\frac{n}{10}$ HCl drop by drop until there is no further precipitation, and the supernatant fluid is clear. Since the precipitate redissolves in acid, use lacmoid as an indicator. Cease when the lacmoid shows a trace of excess. Decant the precipitate, filter, wash the residue with water.

Oxidation by Nucleo-Proteid. — Place in a wide-necked flask 50 c.c. water containing 0.2 gram of the fresh, brown substance and 10 c.c. hydrogen peroxide in a small glass cup. The hydrogen peroxide must be neutralized with from 1 to 1.5 c.c. $\frac{n}{10}$ NaOH. Connect the flask with the lower end of a eudiometer by means of a bent tube. Shake the flask so that the hydrogen peroxide shall come in contact with the tissue. Oxygen is at once set free. Read in the eudiometer the oxygen developed from minute to minute. Spitzer found:

After minutes . .	1	2	3	4	5	8	9	16
C.c. O_2 developed	19	28	41	55	69	85	87	95

Oxidation about the Nucleus.[1] — Introduce the oxidizable solution of a-naphthol and para-phenylenediamine (page 79), beneath the cover glass of a fresh preparation of teased thymus or spleen.

"Granules of the intense greenish-blue oxidation product shortly make their appearance within the leucocytes. Their first appearance is typically at the boundary between nucleus and cytoplasm; eventually the latter may become so densely laden as completely to obscure the nucleus. . . . The nucleus is the chief agency in the intracellular activation of oxygen. The active or atomic oxygen is in general most abundantly freed at the surface of contact between nucleus and cytoplasm."

Glycolysis in Blood. *Bernard's Experiment.*[2] — 125 c.c. dog's blood were divided into five equal parts. The sugar in each was estimated as follows:

	Sugar Grams per 1000.
1. Analysis made at once	1.07
2. " " after 10 minutes . .	1.01
3. " " " 30 " . . .	0.88
4. " " " 5 hours . . .	0.44
5. " " " 24 " . . .	0.00

[1] Lillie: American journal of physiology, 1902, vii, p. 420.

[2] Bernard: Comptes rendus de l'académie des sciences, Paris, 1876, lxxxii, p. 1406.

Sugar disappears from the blood on standing.

It has been found by Lépine and Barral[1] that the glycolytic power of the blood increases as the temperature rises to 52.5° C., which is the optimum. At 54° the ferment is destroyed.

Oxidation not Dependent on Living Cells of Blood. — Place the following solutions at 34–35° C. for six hours, allowing a stream of air to pass through the liquid. Then estimate the sugar.[2]

A. Calf's blood	100 c.c.
Water containing 1.14 gram grape sugar	10 c.c.

Seegen[3] recovered 1.000 gram.

[1] Lépine and Barral: Comptes rendus de l'académie des sciences, Paris, 1891, cxii, p. 146.

[2] Test the filtrate by adding a drop of acetic acid and a little ferrocyanide of potassium.

The absence of a precipitate shows freedom from proteids and ferric salts. Concentrate filtrate to 150–200 c.c.

Titration of the Sugar Extract. — Make the volume of the solution such that its probable content of sugar shall lie between 0.0004 and 0.0010. Causse (Bulletin de la Société chimique de Paris, l, p. 625) recommends that 1750 c.c. of water containing 5 grams of ferrocyanide of potassium be added to each 250 c.c. of Fehling's solution. Boil 10 c.c. of this mixture and add the sugar solution drop by drop until the blue liquid is decolorized (Arthus: Archives de physiologie, 1891, p. 425).

[3] **Estimation of Sugar in Blood.** *Extraction of the Sugar from the Blood.* — To 350–400 c.c. boiling water add *all at once* 50 c.c. blood containing 5 c.c. one per cent acetic acid. Let

B. Calf's blood	100 c.c.
Water containing 1.14 gram grape sugar	10 c.c.
Chloroform	1 c.c.

Seegen recovered 0.960 gram.

The chloroform destroys the cells, but fails to check the oxidation.

Relation of Glycolysis to the Pancreas and the Lymph.[1] — Remove the pancreas aseptically from an anaesthetized dog which has fasted thirty-six hours. Estimate the sugar in the urine at intervals of a few hours.

Sugar will be present in large and increasing quantities,[2] rising even to twenty per cent.

Inject into the jugular vein 15–20 c.c. of lymph from the thoracic duct of a dog fed a few hours before upon one litre of milk.

the mixture boil for a few minutes. Filter through a small linen cloth.

Separation of Proteids. — Boil the filtrate. Most of the proteids will separate by coagulation. The remainder, if necessary, may be removed by adding to each 300 c.c. of filtrate, 5 c.c. saturated solution of sodium acetate, and a small quantity of a dilute solution of ferric chloride, neutralizing almost completely with dilute soda solution, and boiling. The ferric chloride will precipitate as ferrous chloride and will carry down the last traces of proteid substances. Filter. Wash with boiling water. (Seegen: Centralblatt für Physiologie, 1891, v, p. 824.)

[1] Lépine: Comptes rendus de l'académie des sciences, Paris, 1890, cx, p. 742.

[2] Von Mering and Minkowski: Archiv für experimentelle Pathologie und Pharmakologie, 1890, xxvi, p. 371.

The glycosuria will greatly diminish.

After a few hours, the glycosuria will become once more intense, continuing until death. The quantity of sugar in the blood is also greatly increased.

Glycolytic Ferment of Pancreas.[1] — Remove the pancreas aseptically from a dog immediately after death. Crush it at once in 100 c.c. sterile water containing 0.2 gram sulphuric acid. Allow it to macerate two hours at 38° C. Neutralize the acid with soda, add 0.5 gram pure glucose, and keep the mixture one hour at 38° C. Estimate the sugar.

The loss will be from ten to fifty per cent.

When pancreatic extract made without acid is used, the loss of sugar is much less. Probably, therefore, the glycolytic ferment is produced from a zymogen by hydration.

Malt diastase, or salivary diastase, kept three hours at 38° in water containing one tenth per cent sulphuric acid loses the power to change starch to sugar, but acquires a glycolytic power.

If the pancreatic juice which flows upon stimulation of the peripheral end of the vagus (Pawlow) is treated with dilute acid, 1 : 1000, the amylolytic power is lost, but glycolytic power is acquired. During the excitation of the nerve — while the juice is flowing — the

[1] Lépine: Comptes rendus de l'académie des sciences, Paris, 1895, cxx, p. 139.

blood in the pancreatic vein has almost no glycolytic power; after the juice ceases to run, the blood has considerable glycolytic power. Here the external is balanced against the internal secretion of the pancreas.

Oxidative ferments are very widely distributed both in animals and plants. The above experiments show their presence in the blood, pancreas, liver, and lymph. They are present also in the urine.

The stomach contains a ferment that oxidizes lactose to lactic acid.[1]

Urushi, the milky secretion of Rhus vernicifera, dries in the air to a translucent varnish (Japanese lacquer). It contains urushic acid, which does not dry spontaneously, and a ferment, the addition of which to urushic acid causes the latter to dry to lacquer. A sample of fresh juice boiled to stop the action of the ferment on urushic acid contained 15.01 per cent oxygen; lacquer dried in the usual manner contained 20.52 per cent oxygen.

Many oxidations are effected by the tissues without the aid of ferments, so far as is yet known. These belong properly to metabolism, but in passing, it may be noted that while substances exceedingly resistant to oxidation, for example, proteids, are oxidized in the body, other substances very easily oxidizable may be excreted unchanged; oxalic acid is one of these.[2]

[1] Hammarsten : Maly's Jahresbericht der Thierchemie, 1872, ii, p. 118. (Original in Swedish.)

[2] Pohl: Archiv für experimentelle Pathologie und Pharmakologie, 1896, xxxvii, p. 413.

Hoppe-Seyler's Theory.[1] — Living tissues consist of easily combustible reducing substances, which split the oxygen molecules, taking to themselves one atom of O and setting the other free in active state to unite with any oxidizable substance present.

Traube's Theory.[2] — In living protoplasm oxygen is rendered active by an oxidizing ferment, which brings the oxygen to bodies ordinarily oxidizable only by such powerful agents as heat and strong alkalies.

Inorganic bodies, *e. g.* platinum black, the oxides of copper, silver, mercury, and vanadium, and certain iron salts similarly act as oxygen carriers. Thus

$$(1)\quad Pt + O_2 + H_2 = PtO + H_2O$$
$$(2)\quad PtO + H_2 = Pt + H_2O$$

The oxygen carrier reduces H_2O_2, takes one atom O to itself, then gives off this atom in an active or nascent state to oxidize any oxidizable compound present; *e. g.* guiac. Grape sugar takes O from indigo-blue, producing thereby indigo-white. The indigo-white oxidizes itself to indigo-blue, then gives up another atom of O, and so on.

Alcoholic Fermentation

The Yeast Plant. — Observe a solution of sugar undergoing alcoholic fermentation.[3] Note the bubbles of gas, the scum, the sour odor.

[1] Hoppe-Seyler: Zeitschrift für physiologische Chemie, 1879, ii, p. 1.

[2] Traube : Berichte der deutschen chemischen Gesellschaft, 1883, xvi, pp. 123, 1201, and earlier papers in volumes x and xv.

[3] The fermentation is assisted by providing the yeast plant

Examine some of the mixture under the microscope. Note the multitude of globular or slightly ovoid bodies, the largest about $\frac{1}{100}$ mm. in diameter. They are motionless. Many have put forth buds. They seem to be plants in active growth.[1]

1. Place 300 c.c. of the nutrient liquid (Experiment 1) in a flask holding 500 c.c. Add a piece of fresh compressed yeast the size of a pea. Place the flask in a temperature of 35° C.

Note that as fermentation advances the yeast increases in quantity.

2. Place a small piece of fresh compressed yeast in a test-tube. Fill the tube with nutrient liquid and invert it in a dish of similar liquid. The tube may be kept upright by a clamp. Let the mixture stand twenty-four hours in a warm room.

with the salts present in the ash of yeast (Pasteur). A useful substitute is

Potassium phosphate . . .	20 gms.
Calcium phosphate . . .	2
Magnesium sulphate . . .	2
Ammonium tartrate . . .	100
Cane sugar	1,500
Water	8,376
	10,000

(Practical biology, Huxley and Martin.)

[1] Cagniard-Latour : L'Institut, 1835, iii, p. 150 ; also Annales de chimie et de physique, 1838, lxviii, p. 206. The yeast plant was first observed microscopically in beer-yeast by Leeuwenhoek, 1680, but he did not associate fermentation with the growth of the yeast.

The tube will fill with gas. With a bent pipette introduce about 1 c.c. of a solution of sodium hydroxide (sp. gr. 1.12 = 11 per cent). The gas will be absorbed, with formation of sodic carbonate, and the liquid will rise in the tube.

The growth of the yeast plant is accompanied by the production of carbon dioxide.

3. Return to Experiment 1. After the fermenting liquid has ceased to give off gas, place a stopper with a bent tube in the mouth of the flask and distill the contents of the flask in a water bath. Condense the first fifth of the distillate. Saturate this with sodium carbonate. Redistill, and condense.

Test for alcohol by warming the distillate with potassium dichromate and dilute sulphuric acid, whereby the alcohol will be oxidized to aldehyde, with characteristic odor.

Alcohol is present.

The production of alcohol by the yeast is the work of the ferment zymase.[1] This body is closely bound to the protoplasm of the cell, very easily destroyed, not produced in excess, and not secreted free. Only sugars containing three, six, and nine carbon atoms are attacked. The saccharobioses must be "inverted" before they can be fermented. Thus, cane sugar must

[1] Buchner: Berichte der deutschen chemischen Gesellschaft, 1897, xxx, pp. 117, 1110, 2668.

first be inverted to grape sugar by invertin,[1] and malt sugar by maltase. Lactase is present in some yeasts, enabling them to ferment milk sugar. Diastase is also found.

The action of these several ferments becomes clear when the chemical nature of the carbohydrates is recalled.

Chemical Relations of Carbohydrates. — Carbohydrates were formerly defined to be compounds containing six, or a multiple of six carbon atoms, together with hydrogen and oxygen atoms in the proportion in which they exist in water. The researches of E. Fischer have shown that all aldehydes (bodies which are the first oxidation products of primary alcohols, and which contain the carbonyl group CO) and all ketones (bodies which are the first oxidation products of secondary alcohols and which likewise contain the carbonyl group CO) contain carbon, hydrogen, and oxygen, there being two atoms of hydrogen to one atom of oxygen, as in water.

The carbohydrates, therefore, no longer occupy an isolated position, but are to be classed with the fats, being methane derivatives in which the carbon atoms are arranged in an open chain; thus, grape sugar is an aldehyde alcohol, and fruit sugar a ketone alcohol.

The carbohydrates are divided, according to the size of their molecule, into monosaccharides, disaccharides, and polysaccharides. The monosaccharides (*e. g.* grape

[1] For extraction, see Lea : Journal of physiology, 1885, vi, p. 142.

sugar) are the first oxidation products of the hexahydric alcohols; the higher carbohydrates are anhydrides of the monosaccharides. Most of the higher carbohydrates cannot be fermented directly, but must first be hydrolyzed (*i. e.* take up water). This hydrolysis may be accomplished by the prolonged action of dilute acids at high temperatures, by the action of water at still higher temperatures, or by specific ferments, *e. g.* diastase, at the relatively low temperature of the body.

The polysaccharides, consisting of the starches, the gums (*e. g.* dextrine or starch gum) and the celluloses (wood fibre) differ greatly from the lower carbohydrates. The polysaccharides are usually amorphous and are not easily soluble in water.

CARBOHYDRATES.[1]

Glucoses, Monoses $C_6H_{12}O_6$.	Saccharobioses $C_{12}H_{22}O_{11}$.	Polysaccharides $(C_6H_{10}O_5)x$.
Grape sugar—		
Grape sugar—	—Malt sugar—	—Starch
Grape sugar—		
Fruit sugar—	—Cane sugar	
Grape sugar—		
Galactose—	—Milk sugar	
Grape sugar—		—Dextrine

[1] Richter's Organic Chemistry, Third American Edition, i, p. 121.

TABLE ILLUSTRATING THE PRINCIPAL FERMENTS AND THEIR PRODUCTS.[1]

Class of Bodies changed.	Ferment.	Distribution.	Action.
		Actions known or believed to be hydrolytic.	
Carbohydrates	Diastase	Salivary glands. Pancreas. Liver. Intestine. Blood. Lymph. Urine. Seeds. Leaves. Roots. Algae. Mushrooms. Micro-organisms.	Starches to dextrines and maltoses.
	Cytase	Intestine (especially of herbivora). Plant cells. Many bacteria.	Cellulose to reducing sugars.
	Maltase	Salivary glands. Pancreas. Intestine. Liver. Blood. Wide distribution in both plants and animals. Found often with diastase.	Maltose to two molecules of *d*-glucose.
	Invertase	Intestine. Many plants, especially yeasts.	Cane sugar to one molecule of *d*-glucose and one of *d*-fructose.
	Lactase	Intestine. Certain yeasts.	Lactose to *d*-glucose and *d*-galactose.
Glucosides	Emulsin	Almond. Willow. Many other plants.	Amydalin to grape sugar, benzaldehyde and prussic acid.
Nitrogenous bodies	Urease	Micrococcus ureae and many other bacteria. (Compare histozyme, page 64)	Urea to ammonium carbonate. Hippuric acid to benzoic acid and glycin.
	Pepsin	Stomach. Brünner's glands. Urine. Muscles. Saliva. Insectivorous plants.	Proteid and allied nitrogenous bodies to albumoses and peptones.

	Trypsin	Pancreas. Urine.	Proteids to peptones and many fission products; *e. g.*, leucin, tyrosin, asparagin, glutamic acid, hexone bases, glycocoll, tryptophane.
	Rennin	Stomach. Several species of bacteria. Galium verum and several other flowering plants.	Casein to albumose and paracasein (coagulation a secondary process).
	Fibrin ferment	Blood.	Fibrinogen to fibrin (coagulation possibly secondary).
	Myosin ferment	Muscle.	Myosinogen to myosin (accompanying products: lactic acid and carbon dioxide).
Fats	Lipase	Pancreas. Blood. Liver. Many other tissues. Bacteria. Seeds of many plants.	Glycerin esters (neutral fats) to fatty acids and glycerine. (Emulsification a secondary process.)
Ferments known or believed to unite oxygen to the oxidizable substance.			
	Oxidase	Pancreas. Blood. Liver. Lymph. Many other tissues. Many plants.	Aldehyde sugars, etc., to acids and other bodies; *e. g.*, salicyl aldehyde to salicylic acid. Ethyl alcohol to acetic acid. Xanthin to uric acid. Glycogen to sugar.
Grape sugar, etc.	Zymase	Yeasts.	Sugars with 3, 6, 9 carbon atoms to alcohol, carbon dioxide, glycerine, succinic acid, etc.
Proteolytic ferments active in bacteriolysis, agglutination, and haemolysis.			

[1] This table is not intended to classify all known ferments.

The zymase attacks only those sugars which present a specific stereo-configuration. The position of their atoms in space must fit the position of the atoms of the ferment (the lock and the key). Thus, only the dextro-rotatory forms of the aldehyde sugars (d-glucose, d-mannose, d-galactose) are attacked; the sugars that rotate the plane of polarized light to the left are not attacked. It is probable that the zymase of different species of yeast presents characteristic differences. It is known that the products formed in the fermentation of sugar by different species of yeasts are to a large degree characteristic. Often these products are injurious. Upon this specific action of ferments rests the work of Hansen,[1] who taught the brewers to make pure cultures of the most favorable species of yeast, and thereby raised the brewing industry to the level of an applied science.

BLOOD

Specific Gravity

Drawing the Blood. — Wash the lobe of the ear with a bit of absorbent cotton dipped in clean water.[2] Rub the lobe dry with another piece of cotton. Pass a three-sided surgical needle

[1] Hansen: Untersuchungen an der Praxis der Gährungs-Industrie, 1895.

[2] Subjects who are "bleeders" are not to be used for this observation.

through a Bunsen flame. (Do not heat the needle red or the temper will be drawn and the sharpness lost.) Stretch the skin of the lobe between the fingers of the left hand. Make a quick puncture one-eighth inch deep in the edge of the lobe. Press gently to start the flow. The blood must now flow freely. On no account use blood squeezed out.

Determination of Specific Gravity.[1] — Fill a small beaker half full of a mixture of benzol and chloroform of a specific gravity of about 1059. Let a drop of the blood fall into this mixture. The drop will remain spherical, for blood does not mix with benzol and chloroform. If the drop sinks, add chloroform drop by drop, meanwhile stirring the mixture with a glass rod, until the drop neither rises to the surface nor sinks to the bottom but swims with the mixture. If the drop rests upon the surface, add benzol in a similar manner. When the drop neither sinks nor floats, its specific gravity must be that of the benzol-chloroform mixture. Pour the mixture into a glass cylinder, through a piece of linen to hold back the blood-drop, and take the specific gravity of the benzol-chloroform with an areom-

[1] Roy : Journal of physiology, 1884, v, p. ix.

Hammerschlag, A. : Wiener klinische Wochenschrift, 1890, iii, p. 1018.

eter. The result is also the specific gravity of the blood.

The values obtained are slightly too low. The error is one unit in the third decimal place.

Determine the specific gravity of the blood under the following conditions. Record the results in the laboratory note-book. Hand to the instructor a copy of your observations written in ink upon a laboratory blank. The material collected by the class will be analyzed statistically by a committee and a report made.

1. The specific gravity of the blood in a healthy man.

2. In the same man half an hour after drinking 750 c.c. of water.

3. In the same man one hour after drinking 750 c.c. of water.

4. In the same man after profuse sweating. Note any feeling of thirst.

5. In a healthy woman.

Hammerschlag found the specific gravity in chlorosis and nephritis diminished as the haemoglobin diminished. No relation was observed between the appearance of oedema and a reduction in the specific gravity.

Counting the Red Corpuscles. — See that the pipettes of the Thoma-Zeiss apparatus are perfectly clean and dry. Open the bottle contain-

ing Gower's solution (sodium sulphate, 7.3 grams; acetic acid, 20 c.c.; water, 125 c.c.). Prick the ear as directed on page 94. In a large drop which has collected without pressure put the point of the smaller Thoma-Zeiss pipette ("red counter"). Fill the pipette to the mark 0.5 by careful suction. Should the mark be passed, lower the column to the mark by touching the point of the pipette to filter paper. When the mark is reached, clean the outside of the pipette, dip the end in Gower's diluent solution, and draw the liquid very carefully up to the mark 101. (Should the liquid pass the mark, the pipette must be cleaned and dried and the whole process repeated.) Close the ends of the pipette with the fingers, and shake it gently for one minute in order to mix the blood thoroughly with the diluent. The blood will now be diluted 200 times its volume.

Remove the rubber tube from the pipette. Blow out the unmixed solution in the capillary tube, between the point and the bulb, and several drops of the mixture in the bulb. Wipe off the end of the pipette. Touch it to the ruled disc. Let a very small drop flow out. Place the cover-glass on the drop. The flattened drop should almost cover the glass. If it spread into the moat, clean the disc and use a second, smaller

drop. If Newton's color-rings cannot be seen between the coverglass and the disc by placing the eyes near the level of the coverglass, another preparation must be made, with cleaner disc and coverglass.

Use Leitz No. 5 or Zeiss D objective. Bring the drop into focus and then, using the micrometer screw, find the ruled field.

On the central portion of the disc 1 square millimetre has been ruled into 400 squares, each square having therefore an area of $\frac{1}{400}$ square millimetre. Each 16 small squares are surrounded by double lines, thus forming a "large square." In the Zappert-Ewing slide, the central square of 1 mm. is surrounded by eight other squares of 1 mm. each, and the central ruling is extended through the surrounding squares, which are intersected by lines $\frac{1}{4}$ mm. apart. Count the number of corpuscles, square by square, in 200 small squares. Corpuscles touching the north and south lines of each area are to be counted in, those touching the east and west lines are to be omitted from the count.

Each square has an area of $\frac{1}{400}$ square millimetre. The thickness of the layer of blood, *i. e.* the distance from the ruled disc to the coverglass, is 0.1 mm. The volume of the space above each square, therefore, is $\frac{1}{4000}$ cubic millimetre.

As the blood is diluted 200 times its volume, and the number of squares counted is 200, the total number of corpuscles in a cubic millimetre is

$$\frac{x \times 200 \times 4000}{200}$$

x being the total number of corpuscles counted. In short, to obtain the number of corpuscles in a cubic millimetre, multiply by 4000 the number counted in 200 squares. Clean the pipette as soon as the counting is done.

Cleaning the Pipette. — Draw clean Gower's solution through the pipette, then alcohol, and finally ether. Dry the pipette by sucking (not blowing) air through it.[1]

Control Counting. — Count the red corpuscles in a second drop. If the result differ greatly from that of the first count, the corpuscles in a third drop must be counted.

Counting the White Corpuscles. — Have ready a diluting solution of glacial acetic acid (one-third of one per cent). This solution will make the red cells invisible. Obtain a very large drop of blood. Fill the large Thoma-Zeiss pipette (white counter) by very gentle suction. Keep

[1] Pipettes left dirty will be cleaned at the student's expense, or, where necessary, a new one purchased. The cost is considerable.

the pipette nearly horizontal, both in obtaining the drop and in drawing in the diluting solution; the bottle should be tilted. Count the white corpuscles in the entire ruled disc. Repeat with a second drop.

Estimation of Haemoglobin

Hoppe-Seyler's Method[1] (modified). — Weigh about five grams of crystallized haemoglobin. Make a concentrated solution in a measured quantity of distilled water. Saturate with carbonic oxide. Preserve in glass tubes containing about 6 c.c., the ends drawn out and closed in the Bunsen flame. Before using, dilute with distilled water to 0.2 per cent CO-Haemoglobin and saturate with CO. Place in one compartment of the double container with parallel glass sides. In the other compartment place about 2 c.c. of 0.1 per cent sodium hydrate solution. From a drop of blood obtained from the ear as directed on page 94 fill the pipette to the mark (3 cubic millimetres) by careful suction. Should the mark be passed, lower the column to it by touching the point of the pipette to filter paper. When the mark is reached, clean the outside of the pipette, and then blow the contents of the pipette

[1] Hoppe-Seyler: Handbuch der physiologisch- und pathologisch-chemischen Analyse, 1893, p. 412.

into the 0.1 per cent sodium hydrate solution. Remove all the blood by drawing the sodium hydrate solution in and out of the pipette. Look at the parallel columns of haemoglobin solution and blood solution through the blackened tube against an evenly illuminated sheet of white paper placed ten inches away. Dilute the blood with 0.1 per cent NaOH solution until its color is precisely that of the haemoglobin solution.

Measure the volume of the two solutions in c.c. Then the volume of the haemoglobin (a) solution is to the known quantity of haemoglobin which it contains (a') as the volume of the blood solution (b) is to the desired weight of haemoglobin (x) it contains, or the weight of haemoglobin in three cubic millimetres of the blood.

Haemorrhage and Regeneration

Determine the specific gravity, number of red and white corpuscles per millimetre, and percentage of haemoglobin in the same animal under the following conditions: Normal; two hours after a profuse haemorrhage; one day, three days, and five days after the haemorrhage. Plot all three curves upon one co-ordinate system.

Alkalinity

Zuntz-Loewy[1]**-Engel**[2] **Method.** — Place $\frac{n}{75}$ tartaric acid[3] in a finely graduated pipette (1 c.c. in 20) with glass stopcock.

Place in a small beaker 5 c.c. of water distilled in glass and proved neutral in reaction. Select the larger pipette of the Thoma-Zeiss haemocytometer. Note the cubic millimetres contained in the pipette[4] up to the mark 0.5. Remove this quantity from the water. Dry the pipette with alcohol (page 99). Fill the pipette to the mark 0.5 with blood from the ear. Remove any blood from the outside of the tube. Place the end of the tube in the neutral water and gently expel the blood. Wash out the last traces of blood by gently drawing the water in and out of the tube. If this be done with care, the mixture will now measure practically 5.0 c.c. including the blood. Titrate the mixture with the $\frac{n}{75}$ tartaric acid solution drop by drop.

[1] Loewy: Archiv für die gesammte Physiologie, 1894, lviii, p. 462.

[2] Engel: Berliner klinische Wochenschrift, 1898, xxxv, p. 308.

[3] A normal solution of tartaric acid ($C_4H_6O_6$) contains $\frac{48 + 6 + 96}{2} = 75$ g. in 1 litre. A $\frac{n}{75}$ tartaric acid solution contains $\frac{75}{75} = 1$ g. in 1 litre.

[4] This must be determined for each pipette.

Before and after the addition of each drop of the acid let a drop of the mixture fall gently on a piece of light-colored glazed litmus paper previously impregnated with saturated neutral sodium chloride solution. In such paper the salts on which the alkaline reaction of the blood largely depends diffuse more rapidly than the haemoglobin. The latter forms a yellow spot which is surrounded by a clear solution of the blood salts. When the titration acid has combined with all the alkali in the blood a sharp red ring will appear around the yellow spot of haemoglobin. This ring is clearer when the blood is wiped off after having rested a few moments on the paper.[1]

Normally, the alkalinity of 0.05 c.c. blood is satisfied by about 5 c.c. $\frac{n}{75}$ tartaric acid. Then 100 c.c. blood would require $0.5 \times 20 \times 100 =$ 1000 c.c. $\frac{n}{75}$ tartaric acid. 1000 c.c. = 1 litre normal tartaric acid saturates 40 g. NaOH. 1 litre $\frac{n}{75}$ tartaric acid $= \frac{40}{75} = 533$ mg. NaOH. The alkalinity of 100 c.c. blood = 533 mg. NaOH.[2] Hence 0.05 c.c. blood = 0.25 mg. NaOH.

[1] The same tint of paper should be used in comparative experiments. Light colored papers should be observed by direct light; darker papers by transmitted light. Litmus may be replaced by lacmoid (for directions, see Cohnstein: Virchow's Archiv für allgemeine Pathologie, cxxx, and Böckmann: Chemisch-technische Untersuchungs-Methoden).

[2] The average given by Engel is 426.4 — 533.0 mg. NaOH.

Coagulation Time.

Fasten on a glass slide a metal ring pierced with a small hole. Place a little vaseline on the upper surface of the ring. Upon this lay a perfectly clean cover glass upon the middle of the under surface of which has been placed a large drop of blood drawn from the ear with the precautions mentioned on page 94. As soon as the drop is in place within the cell, make it rotate by blowing gently against it by means of a pointed glass tube applied to the hole in the metal ring.

The drop will cease to move when coagulation sets in.

Note the interval between the drawing of the blood and the onset of coagulation.

The method is rough, and a fairly correct result requires much care and a number of observations, but even thus it reveals the important diminution in coagulability in certain diseases, *e. g.* jaundice.

RESPIRATION

Chemistry of Respiration

Estimation of Oxygen, Carbon Dioxide, and Water.[1] — Weigh bottles 3, 4, and 5 (4 and 5

[1] **Apparatus.** — Two aspirator bottles, with box. A wooden tray, containing a jar for the guinea-pig, and six bottles, viz.:

together). Place the guinea-pig in the jar and weigh. During one hour draw air through bottles 1 to 6 by placing an aspirator bottle on its box and allowing the water to flow from this bottle to the one remaining on the desk. The rubber connecting tube must be changed when the aspirator bottles are changed. After one hour weigh bottle 3, and bottles 4 and 5.

Tabulate results as follows:

	Grams.
Weight of jar and guinea-pig at beginning	
" " " end . .	
Loss	
Wt. of bottle 3 (sulph. acid) at beginning	
" " " end . .	
Gain (= water absorbed)	
Weight of bottles 4 and 5 at beginning .	
" " " end . . .	
Gain (= carbon dioxide absorbed) .	
Total water and carbon dioxide absorbed .	
Loss in weight of jar and guinea-pig . .	
Difference (= oxygen absorbed) .	
Respiratory quotient	

Nos. 1 and 4, filled with soda-lime, to absorb carbonic acid; Nos. 2, 3, and 5, filled with pumice stone soaked in sulphuric acid, to absorb moisture; No. 6, a Müller valve, to prevent air being forced back through the series of bottles by a wrong coupling of the aspirator tubes.

Mechanics of Respiration

Artificial Scheme. — Raise the left glass rod above the opening in the rubber tubing. Hold the lower end of the free cylinder even with the rubber balloon, and pour in water till the level just reaches the balloon. Lower the left glass rod to cover the opening.

The surface of the water in the attached cylinder represents the diaphragm and movable chest-walls; the interior of the cylinder above the water, the thoracic cavity; and the rubber balloon, the lungs. The left manometer shows the intra-thoracic pressure; the right manometer shows the intra-pulmonary pressure. The left glass rod closes the entrance to the cylinder, *i. e.* makes the thoracic cavity a closed cavity, as is normal; the right glass rod, with its lower end partly covering the opening in the rubber tubing, controls the entrance to the balloon (the respiratory passages).

Inspiration. — Nearly close the respiratory passage. Lower the water level to the base of the thoracic cylinder.

Note the change in the size of the lung, and in the pressure in the lung and in the thorax. Give reasons for these changes.

Expiration. — Widen the respiratory passage

slightly. Raise the water level slowly till the lung is slightly but evenly distended.

Note the pressure in the pleural cavity. Is it positive or negative? Why?

Normal Respiration. — Slowly and rhythmically raise and lower the diaphragm (water level) between the inspiratory and expiratory level, taking care that the lung never becomes even slightly collapsed at the end of expiration.

Give reasons for the changes in the intra-pulmonary pressure.

Forced Respiration. — Raise and lower the diaphragm more quickly.

Observe that the differences in pressure are increased.

Obstructed Air Passages. — Diminish the inlet in the respiratory tube by moving the glass plug. Raise and lower the diaphragm.

The differences of pressure will be increased.

Asphyxia. — Close the entrance to the lungs entirely.

Note the effect of movements of the diaphragm upon the intra-thoracic and intra-pulmonary pressures.

Coughing: Sneezing. — Remove the glass rod from the respiratory passage. Bring the lung to full inspiration. Close the respiratory opening with the moistened thumb. Raise the diaphragm

half-way toward expiration. Suddenly open the respiratory passage.

Air is quickly and forcibly expelled from the lung (cough, sneeze).

Hiccough. — Lower the diaphragm quickly toward full inspiration, and while the lung is expanding close the respiratory opening with the moistened thumb (hiccough).

Note the sudden changes of pressure in the two cavities.

Perforation of the Pleura. — Open the inlet to the pleura.

Note the effect of the opening into the pleural cavity upon the lung and upon the intra-pulmonary and intra-thoracic pressure.

Observe the result of movements of the diaphragm.