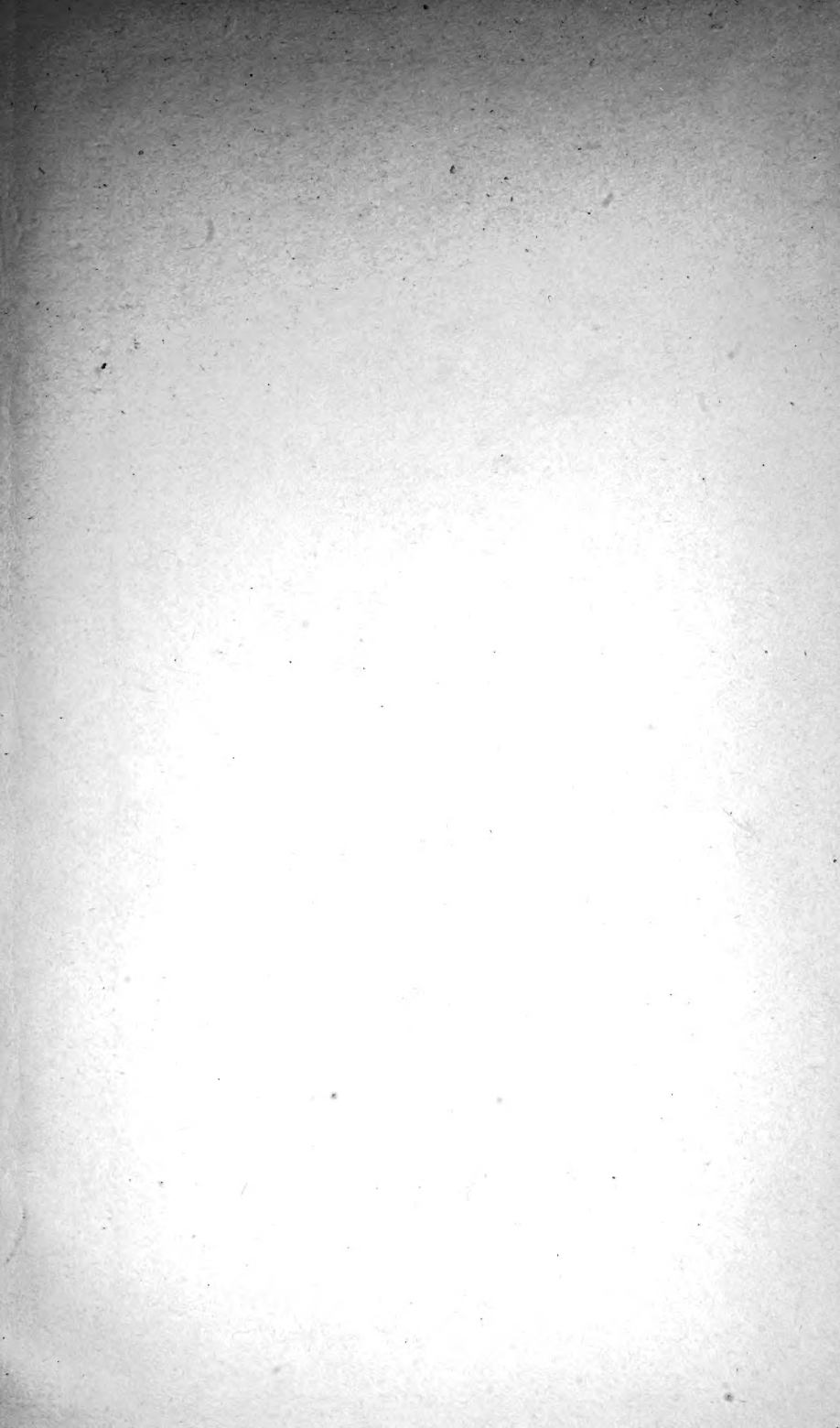





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PRACTICAL PHYSIOLOGY



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# PRACTICAL PHYSIOLOGY

*Edward  
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LONDON  
EDWARD ARNOLD & CO.

1922

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## PREFACE

The Institutes of Medicine, the older and better name for Physiology, are the basis of Medicine; the investigation of the abnormal subject must be founded upon the study of the healthy organism. The importance of practical physiology is undoubted, but there is considerable difference of opinion as to the nature and scope of the experimental work which is most suitable for a medical student. Nevertheless, it is becoming more clearly recognised that the practical work should have a direct relation to medicine and, as far as possible, the experiments should be performed upon man.

In the present book the authors have given a further extension to practical physiology along these lines. They have built largely upon the foundations of the *Practical Physiology* by A. P. Beddard, J. S. Edkins, M. Flack, Leonard Hill, J. J. R. Macleod and M. S. Pembrey. The third edition of that book was exhausted two or three years ago, and most of the contributors are no longer engaged in the teaching of physiology to medical students. The present authors wish to thank them heartily for their kind permission to make full use of their contributions.

The volume is divided into two parts: Part I, Experimental Physiology, Elementary Course by D. Noël Paton, Advanced Course by M. S. Pembrey; and Part II, Chemical Physiology by E. P. Cathcart. The special chapter upon the "Investigation of the Motor Functions of the Alimentary Canal by Means of the X-Rays," which appeared in the third edition of *Practical Physiology*, has been revised by its author, Dr. Hurst; for this valuable contribution hearty thanks are given.

Figures have been borrowed from *The Essentials of Human Physiology*, by D. Noël Paton, and from *The Physiological Action of Drugs*, by M. S. Pembrey and C. D. F. Phillips. For the loan of blocks the authors are indebted to Messrs. Baird and Tatlock, Hatton Garden, E.C., Messrs. William Green and Son, Edinburgh, and Messrs. Henry Frowde and Hodder and Stoughton, London.

The drawings of crystals were executed by Mr. W. R. M. Turtle for the first edition of *Practical Physiology*. The sources of other diagrams and tracings, which have been borrowed, are indicated in the description of the figures. The initials of the author, who took the record of the original tracings, are appended to the respective curves.



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## WEIGHTS AND MEASURES

The unit of the Metric System is the Metre, which represents one ten-millionth part of a quarter of the meridian of the earth. The multiples and subdivisions are obtained by the use of decimals; the former being designated by Greek prefixes, the latter by Latin prefixes.

Metric or Decimal.	LENGTH.	English.
1 <b>Metre</b> (M.) . . . . .	=	39·3701 inches.
1 Decimetre (dm.) . . . . .	=	3·9370 "
1 Centimetre (cm.) . . . . .	=	0·3937 "
1 Millimetre (mm.) . . . . .	=	0·0393 "
1 Micromillimetre (mkm.) . . . . .	=	0·000039 "
1 Myriametre (Mm.) . . . . .	=	6·2137 miles.
1 Kilometre (Km.) . . . . .	=	0·6214 "
1 Hectometre (Hm.) . . . . .	=	109·361 yards.
1 Dekametre (Dm.) . . . . .	=	32·8084 feet.
1 Metre (M.) . . . . .	=	39·3701 inches.
1 inch . . . . .	=	2·539 centimetres.
1 foot . . . . .	=	3·047 decimetres.
1 yard . . . . .	=	0·91 metre.
1 mile . . . . .	=	1·60 kilometre.

Metric or Decimal.	WEIGHT.	English.
1 Kilogramme (Kgm.) . . . . .	=	2·2046 pounds.
1 Gramme (Gm.) . . . . .	=	15·4323 grains.
1 Decigramme (dgm.) . . . . .	=	1·5432 "
1 Centigramme (cgm.) . . . . .	=	0·1543 "
1 Milligramme (mgm.) . . . . .	=	0·0154 "

The unit is the Gramme which represents the weight of a cubic centimetre of water at 4° C.

APOTHECARIES WEIGHT.	
437·5 grains (gr.)	= 1 ounce.
16 ounces (℥)	= 1 pound (lb.)
* { 60 grains	= 1 drachm (℥).
20 grains	= 1 scruple (ʒ).
1 grain	= 0·0648 gramme.

\* Not official.

AVOIRDUPOIS WEIGHT.	
16 drachms	= 1 ounce (oz.).
16 oz.	= 1 pound (lb.).
28 lbs.	= 1 quarter (qr.).
4 quarters	= 1 hundredweight (cwt.).
20 cwt.	= 1 ton.
1 stone	= 14 pounds.
1 pound	= 453·592 grammes.
1 ounce	= 28·35 grammes.

Metric or Decimal.	CAPACITY.	English.
1 Dekalitre (Dl.) . . . . .	=	2·1998 Imperial gallons.
1 Litre (L.) . . . . .	=	35·196 Imperial fluid ounces.
1 Decilitre (dl.) . . . . .	=	3·5196 " "
1 Cubic centimetre (c.c.)	}	= 0·0352 " "
or		
1 Millilitre (ml.) . . . . .		

## WEIGHTS AND MEASURES

## CAPACITY.

60 minims (M) . . . . .	= 1 fluid drachm (3).
8 fluid drachms . . . . .	= 1 fluid ounce (3).
20 fluid ounces . . . . .	= 1 pint (O).
8 pints . . . . .	= 1 gallon (C).
<hr/>	
1 cubic centimetre . . . . .	= 16.9 minims.
1 fluid ounce . . . . .	= 28.42 cubic centimetres.
1 pint . . . . .	= 568.34 cubic centimetres.
1 gallon . . . . .	= 4.54 litres.
1 cubic inch . . . . .	= 16.387 cubic centimetres.
1 cubic foot . . . . .	= 28.317 litres.

## THERMOMETERS.

## FAHRENHEIT AND CENTIGRADE SCALES.

To convert degrees F. into degrees C., deduct 32, multiply by 5, and divide by 9.  
 To convert degrees C. into degrees F., multiply by 9, divide by 5, and add 32.

F.	C.	F.	C.
212°	0.0°	80°	26.7°
112	44.4	70	21.1
106	41.1	60	15.6
104	40.0	50	10.0
102	38.9	41	5.0
101	38.3	32	0.0
100	37.8	23	- 5.0
99	37.2	14	- 10.0
98	36.7	5	- 15.0
97	36.1		

## VARIOUS.

1 kilogrammetre . . . . .	= 7.236 foot pounds.
1 foot pound . . . . .	= 0.1382 kilogrammetre.
1 large or kilocalorie (18°) . . . . .	= 426.6 kilogrammetres.
1 " " " " . . . . .	= 3087 foot pounds.
1 horse-power . . . . .	= 4562.4 kilogrammetres per minute.
1 " " " " . . . . .	= 33,000 foot pounds per minute.

1 gramme Oxygen	at 0° and 760 mm. pressure	= 0.699 litres.
1 " Carbon dioxide	" " "	= 0.508 "
1 " Water vapour	" " "	= 1.244 "
1 litre Oxygen	" " "	= 1.429 grammes.
1 " Carbon dioxide	" " "	= 1.965 "
1 " Nitrogen	" " "	= 1.254 "
1 " Air	" " "	= 1.292 "
1 " Hydrogen	" " "	= 0.900 "
1 " Water vapour	" " "	= 0.8132 "

1 gramme Protein	966.3 cubic centimetres			
	of oxygen intake and	773.9 cubic centimetres of carbon		
		dioxide output.		
1 " Fat	2019.3 " " "	1427.3 " "	" "	" "
1 " Starch	828.8 " " "	828.8 " "	" "	" "

1 gramme urinary Nitrogen . . . . .	= 26.51 Calories.
	= 5.91 litres (8.45 grammes)
	of oxygen.
	= 4.75 litres (9.35 grammes)
	of carbon dioxide.

## AVERAGE WEIGHTS AND HEIGHTS.

Average weight of a healthy male child at birth . . . . .	= 6.8 lbs.
" " " " six months old . . . . .	= 12.4 "
" " " " twelve " . . . . .	= 18.8 "

A healthy adult man (dressed), 5 feet 8 inches in height, weighs about 11 st., and has a chest circumference of about 38 inches.



EXPERIMENTAL PHYSIOLOGY

ELEMENTARY COURSE

BY

D. NOËL PATON



## INTRODUCTION

(To be read before starting practical work.)

Before starting work the student should get a clear idea of the objects of the Course. They are twofold—First, to train him in the investigation of the many problems of medical science which he has afterwards to face, and to teach him to observe, record, and describe the vital phenomena with which he has to deal. Second, to give him a real and sound practical foundation for his after study of Physiology, based upon his personal experience and not upon the dicta of his teacher and Text Books.

For these reasons, the problems to be investigated and the methods of investigation are indicated here; but the results to be obtained and the conclusions to be drawn are left to the student, who must, before all, learn to observe and to experiment with a mind open to accept whatever results may be obtained. From these he should attempt the solution of the problem under investigation.

Throughout this Course the student should keep careful records of every experiment he performs, and these should be made as the experiment proceeds and not some time after its completion. When apparatus is used, he should make diagrams of its arrangement, and tracings must be fixed and preserved. Before beginning an experiment he must first clearly understand its *object*, and enter in his note-book the question to be investigated. He must also, before starting, understand the *method* adopted and how it will throw light upon the question. These methods may be divided into the objective and the subjective. The former attempts to arrange that the processes under investigation, so far as possible, record themselves; while the latter consists in the examination of the sensations of the experimenter himself or of another person as described by that person.

While carrying out the experiment he should not confine his attention only to the main *result*, but should observe everything that happens, and record for further investigation anything he does not understand. An attempt must be made to draw *conclusions from the results obtained*, and to give an answer to the question which is under investigation.

In each experiment the student must record:—1. The Object.

2. The Method used. 3. The Results obtained. 4. The Conclusions drawn.

Every student should be provided with a large note-book, pencil, *strong* sharp-pointed scissors, *strong* dissecting forceps, and a camel's-hair brush.

After completing each set of experiments he should read in a Text Book the results obtained and the conclusions arrived at by others, and by further reading and lectures he should extend the knowledge gained from his own work.

Students who have not made themselves familiar with the electrical apparatus which is used in physiology will find a brief description in the Appendix (p. 77).

The Course consists of seventeen lessons, each occupying about two hours. In some of them it will be found convenient to divide the class into sections, each spending half an hour or an hour on one part of the work, and then exchanging with the other section.

To help the teacher in preparing for the Class, a list of what should be got ready is appended to each lesson.

It may be found of advantage to alter the order of the lessons. If Respiration and Circulation are taken before Nerve-Muscle, a more gradual passage from easier to more difficult experiments is secured. If the Class has not already done physiological work in its Biological Course, it may be advantageous to start with Lesson XVII, § III. If such work has been already done this may be omitted.

## SECTION I

### THE SPECIAL SENSES

#### *HOW DIFFERENT EXTERNAL CONDITIONS ACT UPON THE BODY AND HOW WE GAIN KNOWLEDGE OF OUR SURROUNDINGS*

The body has developed both structurally and functionally to enable the individual and the species to survive in the face of external conditions. Hence its capability of modifying its actions to suit its surroundings is of primary importance, and the ways in which external conditions act upon it to bring about appropriate reactions require special study. The body as a machine liberating energy in its activities is acting in subservience to these influences from without. For this reason alone it is reasonable to begin the study of physiology by the investigation of the various receiving arrangements by which different kinds of external change produce their definite effects either with or without those modifications of consciousness which we call sensations.

Since it is by changes in our consciousness that we are made aware of our existence, and since these changes are brought about by the stimulation of specially-developed receptors, each of which when stimulated gives rise to a particular kind of sensation, the physiological action of these arrangements may be termed the physiology of the Special Senses.

A second advantage in taking the Special Senses as the starting point is that, since the methods employed are largely subjective, the use of complicated recording apparatus is not generally necessary.

A third advantage is that the student early learns the limitations of the senses in gaining knowledge of the outer world, and the danger in interpreting wrongly the sensations experienced. Of all these dangers the greatest to educated people is undoubtedly the element of expectancy with which an observation or experiment is approached. The limitation of our senses must be accepted, but the effects of this element of expectancy can be eliminated, and one of the first things to be learned is to observe honestly and without preconceived ideas.

## LESSON I

## SENSATIONS CONNECTED WITH THE SKIN

Sections of skin showing the epidermis, dermis, papillæ, if possible with tactile corpuscles, and hair follicles, should be studied under the microscopes.

## I. Sense of Contact—Touch

## 1. Is Contact felt equally all over the Surface ?

METHOD.—(1) Fit two or three brush bristles of different strengths into split wooden matches. One student now lays his hand on the table, palm downwards, and closes his eyes. The other touches various points close together over a small part of the back of the hand, about 1 cm. square, and the student experimented upon says whether he feels the contact or not. The points on which contact are most clearly felt are mapped out (Pressure Spots).

(2) He now passes a small fragment of soft cotton wool lightly over different areas of skin, and notes the sensation produced, and whether all parts are equally sensitive.

## 2. What Differences of Pressure can be distinguished ?

METHOD.—One student lays his hand on the table, palm upwards. He keeps his eyes closed while another student applies to the palmar aspect of the proximal phalanx of the middle finger the different weights supplied. The weights must be applied to the same place in the same way each time, and at as nearly as possible equal intervals of time. They must be left on for the same time, not more than about 3 seconds. As each weight is applied the subject of the experiment says "the same," unless he is *sure* that there is a difference, in which case he says "heavier" or "lighter."

Recording the result of each observation, the experimenter then calculates and records the smallest *percentage* difference of weight which can be appreciated with certainty. The weights used are marked in units; their absolute values, within limits, are immaterial.

## 3. Can Points of Contact be discriminated equally well at different Parts of the Surface ?

METHOD.—This may be determined by finding how near to one another two contacts may be made and felt as *two* and not simply as *one* contact.

One student closes his eyes and lays his hand, palm downwards, on the table. The experimenter then takes a pair of compasses, and, holding them loosely in the hand with the points somewhat separated from one another, he lightly brings either one point or the two points simultaneously down upon the back of the subject's hand. The subject must say "one" unless he is *certain* that he feels two points of contact. Working in this way, and recording

the result of each observation as to the distance of the points and the resulting sensation, the experimenter determines and records how far the points must be apart on the back of the hand to give rise with certainty to a double sensation.

The observation is next to be repeated on the palmar aspect of the terminal phalanx of the forefinger and on the dorsal aspect of the forearm.

#### 4. Can Contacts be distinguished however rapidly they follow one another ?

**METHOD.**—Place the finger upon the toothed wheel, first when it is rotating slowly and then when it is rotating rapidly, and note in each case if a *series* of sensations or a continuous sensation is experienced. The contacts are practically instantaneous. What conclusions do you draw as to the duration of the sensations ?

**The Possibilities of Erroneous Interpretations of Tactile Sensations.** (1) *Aristotle's Experiment.*—Cross the middle finger over the forefinger of the same hand and apply a pencil to the adjacent surfaces. Note the character of the perception and the judgment founded upon it.

(2) Dip a finger into the glass of mercury and hold it there for some minutes. Now notice where the sense of pressure is experienced. It is at the surface of the mercury that the finger is subjected to different degrees of pressure.

What do you conclude to be the basis of our sense of pressure ? Is it absolute pressure or differences of pressure at different parts of the skin ?

(3) One student lays his hand palm downwards on the table. His companion now lets the weight provided come to rest first suddenly and then very slowly on the back of his hand. Does the weight feel equally heavy in both cases ?

Is it the actual weight which is appreciated or the suddenness of the change of weight in time ?

## II. Sense of Pain

### 1. Can Pain be produced equally on Stimulating all Parts of the Skin ?

**METHOD.**—With a very fine needle explore the same small part of the skin as in I. 1, p. 6, and map the result. Determine whether the spots, the stimulation of which give rise to the most intense sense of pain, correspond with those giving rise to the most marked sense of touch.

## III. Sense of Temperature

### 1. Is the actual Temperature appreciated ?

**METHOD.**—Take three basins. 1. Fill one with water so warm that the hand can be just comfortably held in it. 2. Fill another with cold water. 3. Fill the third with water at a temperature intermediate between 1 and 2.

Place one hand in 1 and the other in 2, and after keeping them

there for a few minutes place both in 3 and record the sensation in each hand. What conclusion do you draw ?

## 2. What Factors modify the Sensation ?

**METHOD.**—Bring a piece of metal and a piece of flannel, which have been kept at the room temperature, upon the skin and notice the difference in the sensation produced. What is the explanation ?

## 3. Is the Power of Determining Temperature equally distributed over the Skin ?

**METHOD.**—With a cold metal point gently touch the back of the hand at different points between the fourth and fifth metacarpal bones, and notice if the sensation of cold is produced by contact everywhere or only at certain spots.

Repeat the experiment with the metal at a higher temperature than the body.

The experiment may be repeated on the back of the forearm. What conclusions do you draw ?

## 4. What is the smallest Difference of Temperature which can be appreciated ?

**METHOD.**—Take two large test-tubes and half fill them with water at between 35° and 40° C., making one slightly colder than the other. Now find the smallest difference of temperature which can be appreciated (*a*) on the side of the face, (*b*) on the back of the forearm. Start at differences of temperature distinctly perceptible, and add cold water to the warm tube till the difference of temperature can just be detected. Then with a thermometer take the temperature of the water in each tube.

**The Possibility of Erroneous Judgments involving Tactile and Temperature Senses.**—Apply first a cold penny, then a warm penny to the forehead, with the head thrown back, and note whether or not the weight feels the same in both cases.

**Conclusions from the Examination of Cutaneous Sensibility.**—The result of the study of the sensations connected with the skin is to show that there are three kinds of receptors each specially responding to one kind of external change, to one kind of stimulation.—1. Receptors responding specially to contact with gross matter ; 2. receptors responding to injurious stimuli, e.g. the prick of a pin, and 3. receptors responding to the withdrawal or addition of heat.

It appears that these are distributed in a spotted manner over the surface, being more abundant at some parts, less abundant at others.

It is to *changes* in the external condition that they respond, and the suddenness of the change in time or place is the most potent factor.

While each kind of receptor is specially tuned to one kind of change, there is evidence that they may be acted upon by others if these are sufficiently powerful.

(Read Cutaneous Senses in Text Book.)



(If a case of disease of the spinal cord with dissociated sensibility is available, it should be used to demonstrate the conduction from the three kinds of receptors in the skin by separate tracts in the cord.)

*Lesson I. To be provided for the Class.*

1. A small vessel of mercury.—2. A cogged wheel of about 5 inches diameter, with 100 teeth on the circumference, driven by a small motor and with a resistance to vary the speed.—3. A couple of microscopes with sections of skin to show tactile corpuscles and hair follicles.

*To be provided for each pair of Students.*

1. Some brush bristles of different strength fitted transversely in split wooden matches.—2. A very fine steel needle.—3. A small piece of cotton wool.—4. Six small flat pill boxes loaded with shot to weigh about 4–6–8–10–12–14–16–18 gms., and filled up, closed and sealed with paraffin wax. Each should have a distinguishing mark on the bottom, corresponding to a similar mark upon a card with the weight marked upon it for reference.—5. A pair of fine pointed compasses and a millimetre scale. [NOTE.—There is a millimetre scale on the induction coil.]—6. Three basins and a supply of hot and cold water.—7. A piece of metal.—8. A metal rod with a fine point.—9. A thermometer reading to tenths of a degree Centigrade.—10. Two large test tubes.

## LESSON II

### TASTE

Revise the anatomy of the tongue, and examine with a microscope sections of the foliate papillæ of the rabbit's tongue to see the structure of the taste-bulbs.

#### Is the Sensibility to Taste the same all over the Tongue ?

**METHOD.**—Solutions of (1) sugar, (2) quinine, (3) hydrochloric acid, (4) common salt, are provided. One student rinses out the mouth with water, and another applies, with a camel's-hair brush, one or other of the solutions to some part of the tongue and notes the sensation which is said to be produced. The tongue must be kept protruded whilst the taste is being determined. The mouth is again rinsed and the process repeated, and thus the various parts of the tongue are investigated for their sensibility to the different substances. The results should be recorded as a diagram.

#### Is the Sensation of a Specific Nature ?

(a) Tap gently the tip and other parts of the tongue with a clean glass rod and note the nature of the sensations.

(b) Place the free ends of two wires connected with a Daniell cell or the galvanic terminals of the switch board upon the tongue, and record the sensations produced at the anode and kathode respectively at make and break of the constant current.

## SMELL

A bottle of vanilla is opened for a short time in the laboratory where the students are at work, but not to a sufficient extent to enable those at work to detect the smell. They then leave the room and return. Is the odour now perceived? What conclusion may be drawn as to the way in which the sense of smell is stimulated. Is it the amount of vanilla in the air or the change in the amount of vanilla passing through the nose which stimulates?

**The Difficulty of Classification and the Confusion of Taste with Smell.**

—The student closes his eyes, pinches his nose and opens his mouth; his companion places on the tongue pieces of apple. These are replaced in turn by pieces of onion and pieces of potato. The substances may be rolled over the tongue, but should not be chewed, because the texture is different in the three cases. The identification is noted in the several cases and control experiments should be made with the nose open.

Compare the results of this experiment with the so-called loss or impairment of taste during a "cold in the head."

## HEARING

(Revise your knowledge of the physics of sound vibrations and of the anatomy of the ear.)

1. Examine the models of the middle ear, and study the way in which an inward movement of the tympanum is transmitted by the ossicles to the fenestra ovalis. Note especially the joint between the malleus and incus and the way in which it is locked as the tympanum is moved inwards and unlocked as the membrane is moved outwards.

2. Examine a section of the cochlea under a low power and identify the scala vestibuli, scala tympani, scala media, basilar membrane and organ of Corti.

3. Examine a transverse section of the lower part of the Eustachian tube.

4. Close the mouth firmly and hold the nose so as to close the nostrils. Then make a forcible expiration. Note the sensation produced as air is driven up the Eustachian tube.

5. One student closes his mouth. His companion then inserts the nozzle of the Politzer's bag, covered with a small piece of rubber tubing out of a 1 per cent. lysol solution, into one of his nostrils and holds it in position with his finger and thumb, closing in this way the other nostril. He then presses the bag gently and the subject observes if he has a sensation of air entering the drum of the ear.

The subject holds some water in his mouth, and as he swallows it, his companion again compresses the bag with the same force as before, and the subject observes whether air passes into the ear.

What conclusion may be drawn as to the condition of the Eustachian tube during swallowing?

**6. What Qualities of Sounds are Perceived ?**

(i) Loudness. What is this due to ?

(ii) Pitch. What is this due to ?

Is the perception of pitch limited ? Using tuning forks for the lower limit and the steel cylinders supplied for the upper limit, determine the range of perception of musical sounds.

(iii) Quality. (a) Sound the tuning fork *doh* strongly, and note the character of the resulting sensation.

(b) Repeat the experiment, and immediately after sounding the fundamental tone, sound the three partials *me*, *soh* and *doh* above, and note the character of the resulting sensation.

Formulate your conclusions as to the influence of overtones upon the quality of musical tones.

**7. Do Sound Vibrations influence the Internal Ear only through the External Ear ?**

**METHOD.**—Sound a tuning fork lightly, and hold it to the ear until the sound has quite died away. Now place the end of the fork against the closed teeth or the mastoid process. Describe the resulting sensation. What conclusion do you draw ?

Grip your watch between your teeth and close one ear and then the other. Note the sensations. Repeat the experiment with the watch between, but not touching, the teeth. Compare and explain the results.

**8. Is the Power of Localising the Source of Sound well developed ?**

Test the power of localisation by making a faint clicking noise—as by closing sharply a pair of forceps—in the neighbourhood of the head of the subject whose eyes are closed. The latter must make a definite statement as to the direction from which the sound comes.

(Read Physiology of Hearing in Text Book.)

## VOICE

(Revise the anatomy of the larynx.)

**Laryngoscope.**—Fix the mirror of the laryngoscope on the forehead.

A. Examine the model of the larynx by projecting a beam of light into the mouth cavity and inserting the small mirror on the handle into the back of the throat so that the beam is reflected downwards into the trachea. Identify the structures represented.

B. Place a fellow student in one of the stalls in the dark room with a light beside his head, and let him open his mouth widely. Gently warm the small mirror on the handle. Hold down the tongue in a fold of handkerchief. Reflect light into the mouth by means of the mirror on the forehead, and insert the small mirror through the pillars of the fauces and try to see the upper opening of the larynx reflected in it.

Identify the structures seen, and then make the observed person sound a high note, a low note, and take a deep inspiration.

NOTE.—Before inserting the mirror in the mouth of another individual it must be disinfected by plunging for one minute in the lysol provided and then rinsing in water.

(Read Physiology of Voice in Text Book.)

## PROPRIOCEPTIVE MECHANISMS

### I. The Power of Determining the Position and Movements of the Various Parts of our Bodies

#### MUSCLE-JOINT SENSE

METHODS.—With the eyes closed, (a) put the various parts of the arm, hand and fingers in any position, and try if the position of each part can be determined by putting the opposite arm in the same position; (b) get some one to put the same parts in any position, and again try if the positions can be accurately reproduced on the opposite side.

(c) Take some object in the hand and study how an estimate of its weight is arrived at. Has the condition of the muscles, tendons, and joints anything to do with it, and if so, what?

To test the delicacy of this sense find the smallest difference of weight which can be detected, as in Appreciation of Pressure, (p. 6, I. 2), but keeping the hand free of the table and using the muscles of the arm.

(d) By passing the hand over some object with the eyes shut, study how this sense, in conjunction with touch, gives information as to the distance, shape and size of external objects.

### II. The Power of Determining the Movements of the Head in Space

#### LABYRINTHINE MECHANISM

Study a model of the internal ear with special reference to the arrangements of the semicircular canals. Under the microscope study a cross section (1) through an ampulla and (2) through a canal.

Consider the absence of sensation of movement in a smooth-running train, and the sensation of movement on starting and stopping.

1. Spin rapidly round several times, stop and observe the sensation produced.

2. Hold a short stick or poker vertically with its point on the ground. Place the forehead on the top and rapidly walk three times round it. Then raising yourself straight, try to walk to the door. Notice the effect produced and try to explain it.

Try to come to a conclusion as to how the canals may act in the above experiments.

The power of determining the position and movements of the various parts of the body and the head in space, since it depends upon the stimulation of structures by our own position and movement, has been called the Proprioceptive power, and the structures involved may be said to constitute the Proprioceptive Mechanism.

(Read Muscle-joint Sense and Labyrinthine Mechanism in Text Book.)

*Lesson II. To be provided for the Class.*

1. Section of foliate papillæ from the tongue of the rabbit under a microscope.—2. Models of the middle ear and of the internal ear.—3. Section of (a) the cochlea and (b) of the lower part of the Eustachian tube under microscopes.—4. Sections of a semicircular canal and of an ampulla under microscopes.—5. Politzer's bag.—6. A series of tuning forks on sounding boards giving from say 50 vibrations per second upwards; a series of steel cylinders with hammers giving from 8192 v.s UT to 65,536 v.s UT 10, or a Galton's Whistle.—7. Tuning forks on sounding boards giving 512 v.s *doh*, 1280 v.s *me*, 1536 v.s *soh* and 2043 *doh* above.—8. Models of larynx, or a human larynx preserved in formalin and glycerine and split in the vertical mesial plane.—9. Model larynx, laryngoscopes with forehead mirrors, a vessel containing 1 per cent. of lysol and another of water to wash the laryngoscopes.

*To be provided for each pair of Students.*

(Each student must bring a clean camel's-hair paint brush.)

1. In small glasses, solutions of (i) Cane sugar, 2 per cent.—(ii) Quinine sulphate, saturated solution.—(iii) Hydrochloric acid, 0.5 per cent.—(iv) Common salt, 0.5 per cent.—2. Large glass of water for rinsing mouth.—3. Daniell's or other galvanic cell with two wires, or two wires attached to the galvanic terminals of the switch board.—4. Discs of apple, potato and onion.—5. A set of pill boxes with shot weighing about 50, 55, 60, 65, 70, 75 and 80 gms. marked as in Lesson I.

## LESSON III

### VISION

#### A. STRUCTURE OF EYE

##### I. Dissection of the Eye of the Ox or Sheep, fresh or out of Formalin

Examine the eye. Identify the cornea and sclerotic, and notice the entrance of the optic nerve to the inner side of the antero-posterior axis. Note the shape of the pupil and compare it with that of the human subject. Now divide the eye into an anterior and a posterior half by cutting through the equator of the sclerotic with a sharp razor.

Note the gelatinous vitreous humour in the posterior chamber. Note the black-coloured choroid coat inside the sclerotic. In the anterior segment note that the capsule of the vitreous (hyaloid membrane) is firmly attached to the front of the choroid and that it holds the lens in a capsule behind the pupil. Strip the hyaloid membrane and the lens in its capsule from the choroid, and observe how firmly attached it is to a series of ridge-like thickenings of the choroid just behind the junction of the cornea and sclerotic—the

ciliary processes. Examine these processes. Note that the iris is continued forward from them to the edge of the pupil.

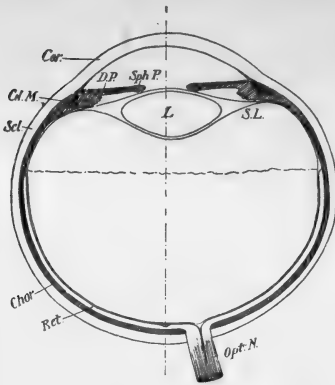


FIG. 1.—Horizontal median section through the eye.

*Opt.N.*, Optic nerve. *Scl.*, Sclerotic. *Cor.*, Cornea. *Chor.*, Choroid. *Cl.M.*, Ciliary process with ciliary muscle. *S.L.*, Suspensory ligament. *D.P.*, Dilator pupillae. *Sph.P.*, Sphincter pupillae. *L.*, Lens.

(From Nöel Paton's Essentials.)

front of the choroid in the eye of the ox. Observe the blood vessels entering in the optic nerve and spreading over the front of the retina. (In the eye of the ox there is no special development of a macula lutea in the posterior optic axis.) Make drawings of the various structures seen.

Study the model of the human eye provided.

## II. Examination of the Eye in Life. Ophthalmoscope

Make a model eye by unscrewing the lower lens of a microscope eyepiece and placing inside it a piece of paper with some mark upon it. Look through the upper lens, and observe that the chamber is dark and the paper is not distinctly seen.

(a) *Direct Method.*—In the optical room fix the model eye in the holder, and bring the electric light beside it so that it does not

Shell the crystalline lens out of its capsule. Study its shape and note its elastic character. Observe the aqueous humour in front of the lens and behind the cornea, filling the anterior chamber.

Now make a section through the cornea and sclerotic at right angles to the last cut. Study and draw the corneo-sclerotic junction with the adjacent tissues.

Examine sections of this part of the eye with the microscope and identify and study the radiating fibres of the ciliary muscle, noting the origin and insertion, and the two sets of muscular fibres in the iris.

In the posterior segment of the eye note the entrance of the optic nerve, and observe the thin membrane-like retina spread over the choroid. Note the iridescence in

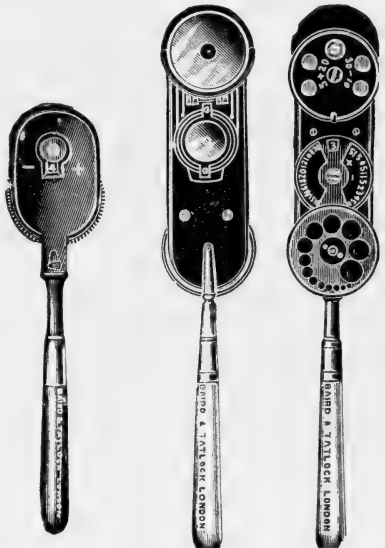


FIG. 2.—Various forms of ophthalmoscope.

shine on the front of the model. Using the mirror with a hole in the centre, reflect light into the model eye, and look into it through the hole. Begin at a distance of 2 feet from the eye, and approach it keeping the light reflected into it. Gradually the mark on the paper becomes distinctly visible. Is it erect or inverted? (*The observer's eye must be kept accommodated for distant vision.*)

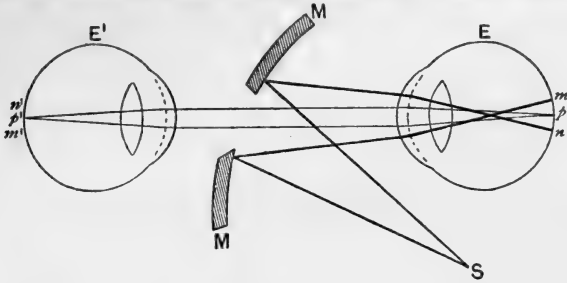


FIG. 3.—Ophthalmoscope—to show the formation of the image in the direct method.

(b) *Indirect Method.*—With the mirror about 3 feet from the model eye reflect the light into it. Now insert a biconvex lens at

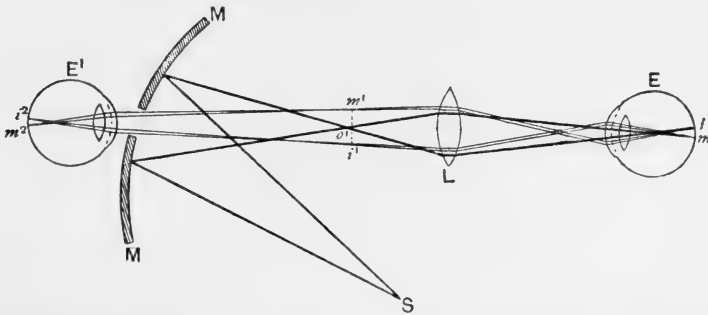


FIG. 4.—Ophthalmoscope—to show the formation of the image in the indirect method.

about 4 or 5 inches in front of the model, and try to see the image of the mark on the paper. Is it erect or inverted?

The human eye may be examined in the same way.

## B. VISION WITH ONE EYE

### I. The Focussing Mechanism

#### 1. The Formation of Images by a Lens.

**METHODS.**—A. In a dark room, using a candle, study the formation of images on an obscure glass screen behind a lens.

1. Where is an object held above focussed on the screen and

where is an object on the right focussed? Is the image erect or inverted?

2. How does the image move with movement of the object?

3. Can a near object and a far object be focussed at the same time?

4. What is the relationship of the size of the image to the distance of the object?

Revise your knowledge of the optical properties of a convex lens, using Kühne's artificial eye or some substitute.

B. Examine the image of a candle formed on an obscure glass plate placed over a hole cut in the back of a *fresh* eye of an ox.

## 2. At what Surfaces of the Eye are the Rays of Light Refracted?

Note the formation of an image from each of the two refracting

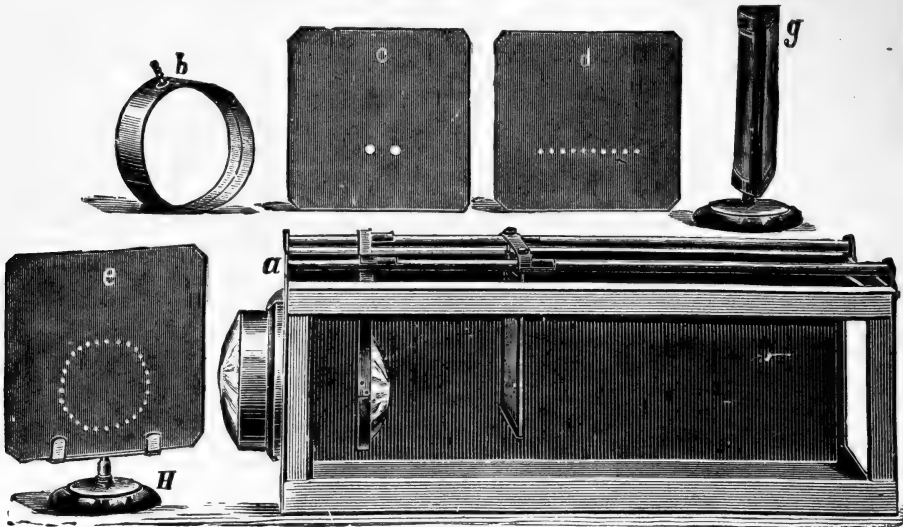


FIG. 5.—Kühne's artificial eye and accessory parts.

surfaces of a biconvex lens due to the reflection which takes place at these surfaces. Study (1) how the size of the image varies with the curvature of the lens, (2) the position, erect or inverted, of the image from each surface of the lens, and (3) the direction in which it moves when the object is moved.

METHOD.—In a dark room hold a candle to the outer side of the eye of a fellow student, and notice that three reflected images are to be seen—one large, clear, distinct, erect image from the anterior surface of the cornea, one small, distinct, inverted image from the posterior surface of the lens, and one much less distinct erect image, larger than the first and apparently lying almost behind it and seen best from the side away from the light, from the anterior surface of the lens—**Sanson's Images**. From the size of these images draw



conclusions as to the relative curvatures of the different surfaces.

The bright clear erect image is formed from the anterior surface of the cornea. The small sharply-defined inverted image is formed from the back of the crystalline lens. The large, dim erect image which seems to lie behind the first is formed from the anterior surface of the crystalline lens. These, then, are the three refracting surfaces of the eye at which rays of light are bent so that they may be focussed on the retina.

Why is no image formed from the posterior surface of the cornea ?

The refractive indices of the media are :

Cornea	.	.	1.33	Lens	.	.	1.44
Aqueous	.	.	1.33	Vitreous	.	.	1.33

From the results of these observations, make a diagram of the *physiological* lens of the eye.

**3. Can Near and Far Objects be seen at the same Time ?**

**METHODS.**—(i) Close one eye and fix the other on the far corner of the room, and then hold up a pencil at about a foot from the eye and see if *at the same time* both objects can be distinctly seen. Another student should note any change in the pupil when the eye is directed to the pencil.

(ii) *Scheiner's Experiment.*—Make two holes in a horizontal line in a sheet of paper so near that they both fall within the diameter of the pupil. Now stand at about two or three yards from a wall on which a small vertical line is drawn and look at it through the holes. While keeping the eye fixed on the line, bring a needle vertically in front of the holes at about 8 inches from the eye,



FIG. 6.—To show the formation of images in Scheiner's Experiment.  
(From Noël Paton's Essentials.)

and note the appearance of the needle when the distant line is looked at, and of the line when the needle is looked at.

Make a diagram of the experiment and formulate the conclusions to be drawn.

**4. Is the Power of Focussing Limited or Unlimited ?**

**METHOD.**—Bring the point of a pencil held vertically nearer and nearer to the eye; a point is reached within which it cannot be distinctly seen—the **near point**. Measure the distance of this from the eye and record it.

**5. What Change takes place in the Eye in Near Vision ?**

**METHOD.**—Repeat the experiment on the refracting surfaces of the eye (p. 16, I. 2), when the observed eye is looking at a distant and at a near object.

Examine again, using a Phakoscope as demonstrated. (Figs. 7 and 8.)



FIG. 7.—The phakoscope.

Make a diagram of the results arrived at. Which refracting surface is changed?

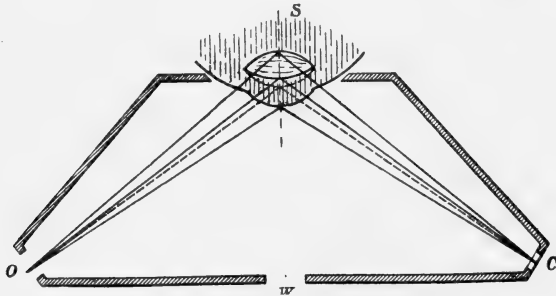


FIG. 8.—Diagram of the course of the rays of light in the phakoscope.

This experiment shows that neither the anterior surface of

the cornea nor the posterior surface of the lens undergo any change in curvature. The image from the anterior surface of the lens becomes smaller and seems to approach that from the cornea. It is the anterior surface of the lens which becomes more convex and thus brings the

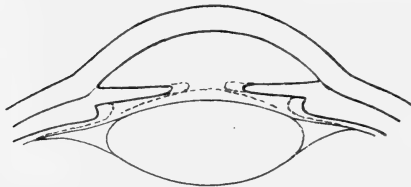


FIG. 9.—To show the changes in the lens and in the pupil in positive accommodation. (From Noël Paton's Essentials.)

more divergent rays from objects near the eye to a focus on the retina.

A knowledge of the origin and insertion of the ciliary muscle explains how this change is brought about.

**Imperfections in the Dioptric Mechanism of the Eye.**—Use some modification of Kühne's artificial eye with a candle about 3 feet

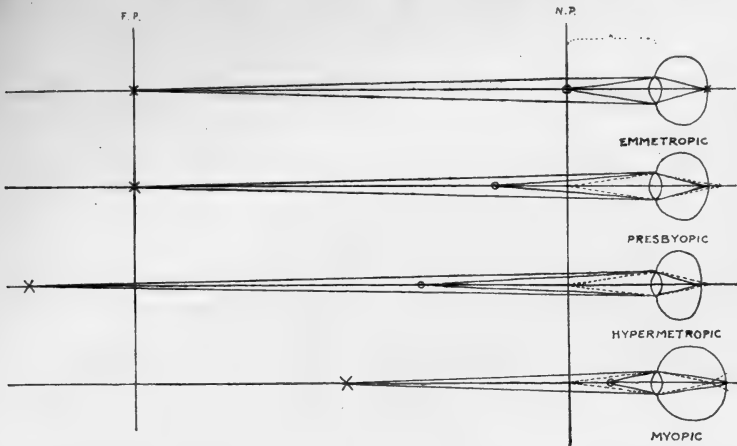


FIG. 10.—To show the way in which rays of light from the far point and near point of vision are focussed in the normal (Emmetropic), in the old sighted (Presbyopic), in the long sighted (Hypermetropic), and in the near sighted (Myopic) eye.

(From Noël Paton's Essentials.)

in front of it so that nearly parallel rays enter the eye. Adjust the screen so as to get a clear image of the candle. Now by moving the screen study the effect of making the eye (1) too long and (2) too short upon (a) the image formed, (b) the distance to which the candle must be moved to give a clear image. Which condition gives short-sightedness (*Myopia*) and which long-sightedness (*Hypermetropia*)?

Using Clark's model with (1) a cylindrical lens convex from above downwards, (2) and one convex from side to side, throw the image of a cross upon the screen. Note the character of the image. Now slide (2) in front of (1) and note the change in the image.

This represents in an exaggerated form the result of any of the refracting surfaces of the eye having its curvature unequal in different planes. This is the condition of **astigmatism**.

Using the card provided, test your eye for astigmatism. If this

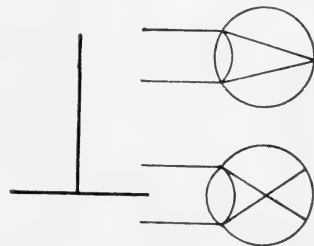


FIG. 11.—To show that in the astigmatic eye rays from vertical and horizontal lines are not focussed simultaneously upon the retina.

(From Noël Paton's Essentials.)

is present all the lines will not be seen with equal distinctness at one time.

(Read the Physiology of the Dioptric Mechanism in a Text Book.)

## II. Action of the Retina and Brain

### 1. Are Visual Sensations Produced by Light only ?

Press upon the eye-ball far back, and note the effect of such mechanical pressure on the retina. Formulate your conclusion.

This is one of the most important experiments in Physiology, for from it may be deduced the conclusion that the stimulation of a receptor by any kind of change produces the same *kind* of sensation, and the converse that the same kind of stimulation of different kinds of receptors will produce different and specific changes of sensation.

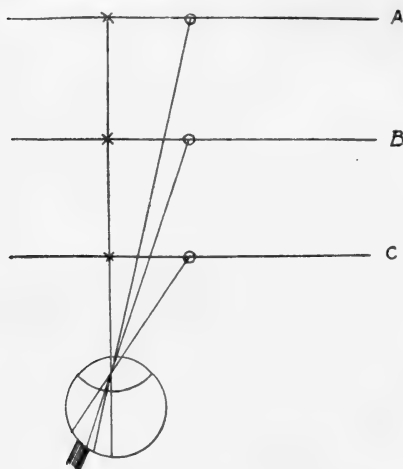


FIG. 12.—To show the demonstration of the Blind Spot by method 2 (i).

(From Noël Paton's Essentials.)

Make a diagram of the results of the experiment.

(ii) Make a mark on the left side of a piece of plain paper. Holding the head *firmly fixed at about a foot from the paper* and closing the left eye, keep the right eye *fixed* upon the mark, and move the point of a pencil, held nearly horizontally, slowly towards the right side of the paper. Note any change in the appearance of the point of the pencil you may observe.

Make a diagram of the experiment and draw your conclusions.

(iii) Map out the blind spot by moving the point of the pencil

This is the law of Specific Nerve Energy.

### 2. Is the whole Retina Stimulated by Light ?

**METHODS.**—(i) *Mariotte's Experiment.* Make two marks horizontally about 4 inches apart upon a piece of plain paper.

With the head about 18 inches from the paper and with the left eye closed, *fix* the right eye on the left-hand mark.

Are both marks visible ? Does any change take place as the paper is gradually brought towards the face ?

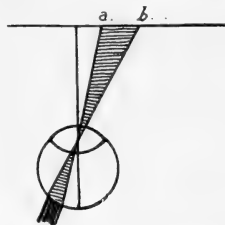


FIG. 13.—To show the demonstration of the Blind Spot by method 2 (ii).

(From Noël Paton's Essentials.)

from the part of the paper where it is invisible to where it becomes visible. Mark the limits of the invisible area in all directions from its centre.

Examine the retina in the model eye, and note that the blind area discovered corresponds in position with the entrance of the optic nerve (p. 14, II.).

**3. Is the Power of Localising the Source of Light equally developed all over the Retina ?**

**METHOD.**—Prepare an experiment as above, but instead of a pencil take a pair of compasses. Bring the points close together, and place them on the central mark, and then note whether both can be seen. Now, keeping the head perfectly steady and the eye fixed on the central mark, draw them along the paper away from the central mark, and note when two points can no longer be distinguished. Separate them till they are again seen as two and draw them still further out, and note what happens. Record the result on a diagram and formulate your conclusions.

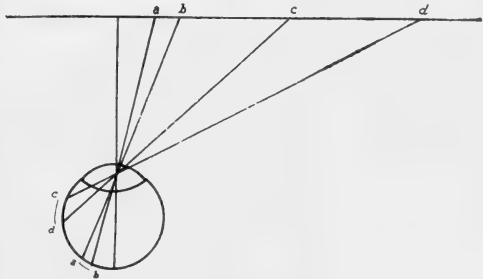


FIG. 14.—To show the decreasing power of localisation on passing from the centre to the periphery of the retina.

(From Noël Paton's Essentials.)

In a good section through the central spot of the retina it may be noticed that, while at that point cones alone exist, further out upon the retina these are situated at greater and greater distance with rods between them. Apparently it is necessary that two cones should be stimulated to get a double sensation.

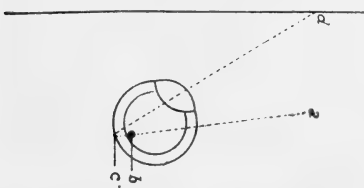


FIG. 15.—To show how shadows of the blood vessels are thrown on the layers of rods and cones in the experiments of Purkinje's images.

(From Noël Paton's Essentials.)

**4. What Layer of the Retina is acted upon by Light ?**

**METHOD.**—In a dark room stand side on against a uniformly coloured wall, with the eyes turned towards the wall. By means of a lens another student directs a powerful ray of light through the exposed sclerotic coat of the eye, and, on moving the light up and down, and from side to side, any appearance on the wall is noted.

The lines seen are shadows of the retinal blood vessels—**Purkinje's Images**. Revise your knowledge of the distribution of the blood

vessels of the retina (p. 14) and draw your conclusions. Is it the front layer of the retina or a back layer which is acted upon by light ?

The following is another method of performing this experiment.

Remove the objective from a microscope, arrange the mirror for a good light and move the microscope from side to side ; a number of vessels will be seen running vertically. The microscope is now moved from before backwards until vessels are seen running horizontally. Give the microscope a circular movement, the field will be covered with vessels except in the direct line of vision in which is a small area in which no vessels are seen, the macula lutea or yellow spot. Make a diagram of the appearances in each case.

*Lesson III. To be provided for each pair of Students.*

1. An eye from an ox, sheep or pig cleared of external tissues, fresh or fixed for a day or two in formalin and then washed. A sharp razor.—2. Two or three ophthalmoscopes and the usual biconvex lenses.—3. A model eye or the eye-piece of a microscope with a piece of paper fixed under the lower lens with some prominent figure X or O marked upon it, the whole fixed in a retort holder beside a suitable electric lamp.—4. Candle in holder.—5. Convex lenses of different curvature.—6. Sheets of paper.—7. Needle in handle.—8. Pair of fine pointed compasses.—9. Millimetre measure.—10. Card for astigmatism.

*To be provided for Class.*

1. Kühne's artificial eye or some similar model.—2. Model of eye in orbit.—3. Microscopes with sections of (a) Anterior segment of eye with ciliary processes ; (b) Posterior segment of eye with retina.—4. A small photographic camera.—5. Phakoscope.

## LESSON IV

### 5. What Range of Objects can be seen at one Time ? The Field of Vision.

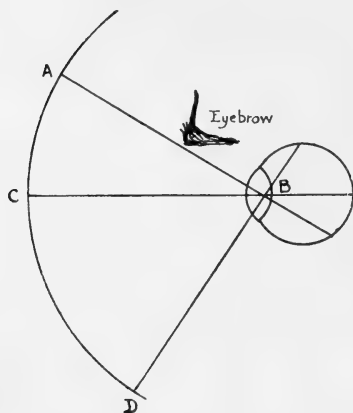


FIG. 16.—To show the Field of Vision in the vertical plane.

(From Noël Paton's Essentials.)

#### METHOD.—A. *Black and White.*

Describe a semicircle on a black-board with the free ends of the line finishing at one side. Mark the centre of the circle at the edge of the board and the middle point in the circumference with an X. This forms a rough "Perimeter."

Keeping one eye closed, the observer places his other eye at the centre and directs it steadily towards the middle point in the circumference. A fellow student slowly draws a piece of chalk along the circumference from below. The subject states when it becomes visible, and the other marks this point.

Now, starting from above, the experimenter again draws the chalk along the circumference until it becomes visible, and marks the point where it comes into view. The angle thus formed with the centre of the circle and these points subtends the vertical field of vision. Measure this angle and record it.



FIG. 17.—A perimeter.

Now turn the blackboard into the horizontal plane, or use the top of the table, and map out the horizontal field of vision.

Why are the angles subtended not equal in the four segments of the field of vision? Will the position of the eye in relationship to the eyebrow and nose explain this?

**B. Colours.** Using coloured papers, map out the field of vision for the different colours, red, green, blue and yellow, noting the points at which *the colour* becomes clearly distinguishable. Measure and record the angles. Draw a section of the eye and mark upon it the parts of the retina which react to black and white, red, green, yellow and blue.

Study the more elaborate instrument, the *Perimeter*, used for making an accurate map of the visual field.

(Read these subjects in Text Book.)

### 6. How are Colours Perceived ?

(Revise your knowledge of the physical nature of colour. Study the spectrum produced by a prism.

#### i. How are the various Colours in Nature produced ?

**METHODS.**—(1) Fix a disc of pure spectral colour, e.g. red, on the rotating wheel in a good light, and after rotating the disc and observing it, introduce, by means of the slit, (a) a segment of white, rotate and observe; (b) a segment of black, rotate and observe; (c) a large segment of bluey-green, rotate and observe. Record your results, and draw conclusions as to the effect upon the colour sensation of mixing a spectral colour with (a) white (diluting it), (b) black (decreasing the illumination), and (c) another part of the spectrum.

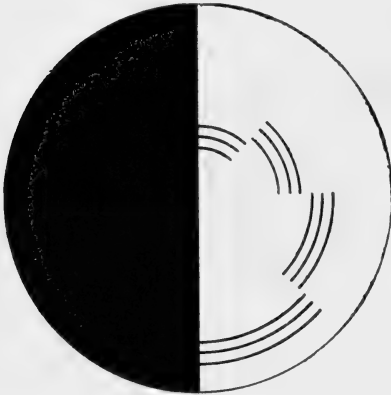


FIG. 18.

#### ii. Are Colour Sensations produced only by Ethereal Vibrations of Different Lengths ?

**METHODS.**—(1) Insert the tip of the little finger into the external angle of the eye, getting it as far back as possible and turning the eye inwards. Now press, and notice if any colour sensation is produced.

(2) Fix the special black and white disc provided on the wheel and rotate at various speeds. (Fig. 18.) Note the effects produced. What conclusion do you draw ?

#### iii. Can all Individuals distinguish Colours equally well ?

**METHOD.**—(1) Take a set of Holmgren's wools. Give a student a red wool and let him pick out all that are of the same sort of colour. Find if any member of the class is colour blind.



(2) Edridge Green's test. Using these cards test your colour visions as directed.

C. VISION WITH TWO EYES

I. What are the Advantages ?

1. Extent of Field of Vision.

Using the top of the table (p. 22, 5), investigate the field of vision in the horizontal plane first for the right eye, then for the left eye, and then for the two eyes together, taking care not to move the head. Make a diagram of the result, and compare the optical angle in vision with one and with two eyes.

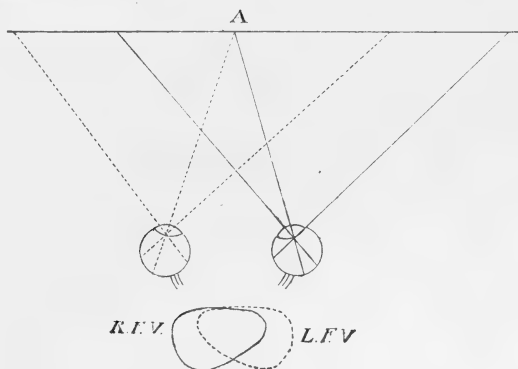


FIG. 19.—To show the Field of Vision for each eye and for the two eyes in the horizontal plane.

A, The point looked at. R.F.V. and L.F.V., the right and left field of vision showing the overlap. (From Noël Paton's Essentials.)

2. Estimation of Contour.

(a) With the **Stereoscope** study how the projection of slightly different pictures on the two eyes gives the idea of relief.

(b) Lay a prism edge on to you on the table, look at it first with one then with two eyes, and consider how the idea of relief is arrived at.

3. Estimation of Distance.

Thread an ordinary sewing needle, keeping both eyes open. Now repeat the process with one eye closed. Can it be done with equal readiness ? What conclusion do you draw ?

*Lesson IV. To be provided for each pair of Students.*

1. A blackboard on an easel.—2. Small goniometer made of cardboard.—
3. Discs of coloured paper, about  $\frac{3}{4}$  inch in diameter, true spectral blue, yellow, red, green and white.

*To be provided for Class.*

1. Perimeter with series of cards.—2. Discs of pure spectral colours

slit to the centre on one side for fitting to the axle of the motor.—3. A small motor with resistance to vary speeds to carry the discs, or a hand-driven disc.—4. Holmgren wools.—5. Edridge Green's card tests for colour blindness.

## LESSON V

### II. Why is there Normally Single Vision with Two Eyes?

#### 1. Is Single Vision possible if the Eyes do not move together?

**METHODS.**—1. With the tip of the finger fix one eye in its socket and move the head about, looking at external objects, and notice whether they remain single.

2. Looking straight forward, press with a finger upon one eye to alter its direction, and note the effect upon vision and draw your conclusions.

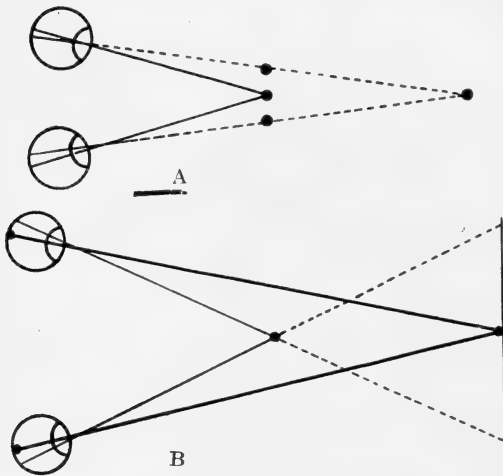


FIG. 20.—To show (A) how on looking at a near object the image of a far object may be doubled, and (B) how on looking at a distant object the image of a near object may be doubled.

(From Noël Paton's *Essentials*.)

action of each of the three pairs of muscles upon the direction of the pip or pupil.

#### 2. Can Double Vision be produced when the Eyes move freely together?

**METHOD.**—Set up a stick vertically about 3 feet from the eyes, and another at about 10 feet. Look at the near one and see what happens to the image of the far one. Close one eye and observe what happens. Now look at the far one and notice the image of the near, and again close one eye. Make a diagram of the experiment and explain the result. (Fig. 20.)

(Read *Binocular Vision* in Text Book.)

Study the anatomy of the eye in the orbit, the direction of the axis of the eye and that of the orbit, and the action of various muscles which move the eyeball. Note that they act round three axes of rotation. Now get an orange and take the pip to represent the pupil, or use a ball of wool, with some mark to represent the pupil. Thrust a needle through each of the three axes of rotation and study the

*D. MODIFIED VISUAL PERCEPTIONS***1. Effects of Strong or Prolonged Stimulation.**

**METHODS.**—(a) After keeping the eyes closed for two minutes look steadily at a white mark on a black surface for a few seconds and then close the eyes.

Describe the image that appears and also any change in its intensity as time passes—Positive After-Image.

(b) Look steadily at the same mark for three minutes and then close the eyes.

Describe the image as above—Negative After-Image.

**2. What is the Effect of prolonged Stimulation of the Eye with any one Part of the Spectrum ?**

**METHOD.**—Put a disc of red paper upon a white ground in a strong light. Look steadily at it for half a minute, then remove it and continue to look at the white surface, and note what happens. Repeat this with discs of different colours.

The colour which appears is said to be complementary to the first.

**3. The Possibility of Erroneous Interpretation of Visual Sensations.**

1. Two squares of equal size are fixed upon paper : one is white placed upon a black ground, and one black placed upon a white ground. Which appears larger ?

2. Place three equidistant dots in a straight line on a piece of paper and subdivide one division by a series of dots. Which part appears longer ?

3. (a) A red square is placed on a white ground and another on a green ground. Which appears redder in colour ?

(b) A red paper is placed on the table with a grey one a foot away on one side and a green one a similar distance away on the other.

Does the red appear redder after looking at the grey or at the green sheet.

4. Make two marks on a sheet of paper as in Mariotte's Experiment (p. 20, II. 2) but at about  $1\frac{1}{2}$  to 2 inches apart, or draw a cage in the position of one mark and a bird in the position of the other. With both eyes open hold the paper near the near point of vision and then focus the eyes for a distant object through the paper. Note what seems to happen to the marks or to the bird and the cage. Try to explain this. The rays from a distant object are practically parallel and falling on the central spots of the two eyes give the idea of a single object. When by this artifice the image of the bird and of the cage fall upon the two central spots they are mentally referred to the same place and seem to coincide.

5. Rule a square with parallel diagonal lines, and place short vertical and horizontal lines upon the alternate diagonals. Do the latter now appear parallel ?

From these experiments draw your conclusions as to the necessary accuracy of the knowledge gained by vision.

(Read Modified Visual Perception in Text Book.)

### E. TIME TAKEN IN VISUAL PERCEPTION

(The method of driving the recording drum, of varying its speed and of altering its direction, of fixing and of smoking the glazed paper should be demonstrated.)

METHOD.—Connect with the bench terminals marked *F*, or with a dry cell in one circuit, an electro-magnetic time-marker *C*, and with two mercury keys, *A* and *B*, separated by a considerable length of wire, so that when both keys are closed the current passes and the lever on the marker is depressed. Bring the points of the lever of the marker *C* lightly against the smoked surface of a rapidly revolving drum. Key *B* being open, one student stands beside the drum and lever watching this lever, and holding the handle of the closed mercury key, *A*, which he must open the moment he sees the lever depressed. Another student now closes the key, *B*, in such a way that the subject can neither see nor hear the closing. The lever is thus suddenly depressed. It is released again when the first student opens *A*, when the point of the lever will spring up. Below the record thus obtained, a time tracing in  $\frac{1}{100}$  sec. is made. To do this, set the drum going, and, when it is revolving uniformly, strike a tuning fork vibrating one hundred times per second on the thigh so as to set it vibrating and holding it by the handle, and with the two limbs in the vertical plane, bring the wire attached to one limb against the drum under the trace and run off a time trace. Each tooth on the tracing represents  $\frac{1}{100}$  second.

The interval between the application of the stimulus and the resulting action is measured and recorded. At least two observations must be made, and unless the time measurements correspond closely a third must be carried out.

After writing with a pin or other instrument name, date, and nature of experiment, the tracing is fixed by passing it through varnish and hanging it up to dry. *Preserve the trace and measurements in your note-book.*

A similar method may be used for measuring the time taken in perceptions with the other senses.

#### *Lesson V. To be provided for each pair of Students.*

1. Discs of paper of pure spectral colours.—2. Sheets of white paper.—3. Black square on white paper, and white square of same size on black paper.—4. Red square on white paper, and similar red square on green paper.—5. Electro-magnetic time-marker.—6. Recording drum covered with smoked paper.—7. Two mercury keys.—8. Lengths of insulated copper wire of about 6 feet.—9. A tuning fork vibrating 1-100th second with marker on one limb.

#### *To be provided for the Class.*

Perimeter; Stereoscopes with some suitable figures.

## SECTION II

# NERVE AND MUSCLE

### LESSON VI

The ways in which the outer world acts upon us through our various receptor mechanisms having been studied, the problem of how we react through our neuro-muscular mechanism may next be investigated. The influence of the brain and of the spinal cord upon the muscles and the way in which the nerves act upon them may first be studied.

This may be done by comparing the condition of a frog with the brain intact with its condition after the brain has been destroyed. The spinal cord is then the only part of the central nervous system connected with the muscles and its influence may be determined by observing the condition before and after it is destroyed.

Revise your knowledge of the anatomy of the brain, spinal cord and nerves of the frog. With a microscope examine (1) a piece of nerve teased in 0.75 NaCl solution; (2) a transverse section of a nerve stained with osmic acid, and (3) a transverse section of the spinal cord stained by Weigert-Pal's method.

#### A. THE INFLUENCE OF THE BRAIN ON THE SKELETAL MUSCLES

Arrange an induction coil for stimulation with Neef's hammer (see Appendix, p. 81).

METHOD.—Study, draw and describe the attitude of the frog supplied, and note the movements and the effect of touching and of turning the animal on its back—

(1) *With the brain and spinal cord intact.*

(2) *After the brain has been destroyed.* Hold the frog by the hind legs in the fold of a towel and kill it by hitting the edge of the table a sharp blow with its head. Then cut off the head behind the tympanic membranes. After a few minutes, study and describe the attitude, the movements, and the effect of touching and pinching. Feel the condition of the muscles as to consistence, and place the animal on its back noting any difference in its behaviour.

What is the influence of the brain upon the muscles and upon the power of balancing?

**B. THE INFLUENCE OF THE SPINAL CORD ON THE SKELETAL MUSCLES**

**I. Reflex Action in the Frog**

**1. Phenomena of Reflex Action.**

Now hang the frog to the edge of the frogboard, fixing it by a pin through the jaw. Pinch the toes of one foot and observe and record what happens. This is a reflex action.

1. *Are reflex movements co-ordinated?* Apply a *very small* scrap of blotting paper dipped in acetic acid to the flank of the animal. Study and describe the movements.

Having washed off the acetic acid, study—

2. *The relationship of the reflex response to the stimulus.* Pinch the foot with forceps and study the result as regards—

(a) Movements which result. (Describe.)

(b) Relation of these movements to the strength of the stimulus. Vary the strength of the pinch, or, stimulating with the induced current (Neef's hammer, see Appendix, p. 83), vary its strength.

(c) Duration of the movements. How long are the movements maintained with different strengths of stimulus?

(d) Spread of the movements. Study the order of this.

3. *What is the effect of a series of subminimal stimuli in liberating a reflex action?*

(A stimulus which is too weak to cause a reaction is called a *subminimal* stimulus.)

Stick the pin electrodes lightly into the skin of the foot. Withdraw the secondary coil of the inductorium till a *single* stimulus just fails to elicit a response, then stimulate the foot with—

1. A single subminimal stimulus.

2. A series of subminimal stimuli.

What conclusion do you draw?

**2. Is Time taken in Reflex Action?**

METHOD.—Using the same frog, dip the foot first into the weak acid supplied, and, after washing it by immersing in a vessel of water, into the stronger acid. Record the difference in the time of onset of the reflex action.

**3. Fatigue.**

Arrange as in 3 above, with the secondary coil pushed nearer the primary.

Continue a series of fairly strong stimuli till the leg no longer responds—till fatigue is produced. Now dissect out the sciatic nerve. To do so snip through the skin on the dorsal surface of the thigh, then, holding the thigh between the thumb and forefinger of the left hand, separate the flexors and extensors and with a glass hook draw forward the thin biceps muscle and the white nerve will be clearly seen and may be separated from the surrounding

muscles by the glass hook so that the electrodes may be inserted under it. Now bring the electrodes under it and stimulate it. Note the result and draw your conclusions as to—

1st. Where fatigue manifests itself. Is it in the nerve or in the spinal cord ?

2nd. The action of nerve when stimulated upon skeletal muscle.

#### 4. Is Reflex Action Dependent on the Spinal Cord ?

Pith the frog by passing a thick pin down the vertebral canal so as to destroy the spinal cord (Fig. 21).

Note carefully anything that takes place in the muscles as this is done. Study and describe the frog again as to attitude, movements, effect of touching and pinching. Examine the consistence of the muscles and investigate for reflex acts.



FIG. 21.—Method of pithing a Frog.

What is the influence of the spinal cord upon the muscles ?

### II. Reflex Action in Man

#### 1. The Knee-jerk.

One student sits with the right leg crossed on the left, closes his eyes and firmly clasps his hands together. Another student strikes the ligamentum patellæ of the right leg with the edge of the ear-piece of a simple stethoscope or with the side of the hand, and observes the contraction of the quadriceps extensor femoris and the movement of the leg. (Fig. 22).

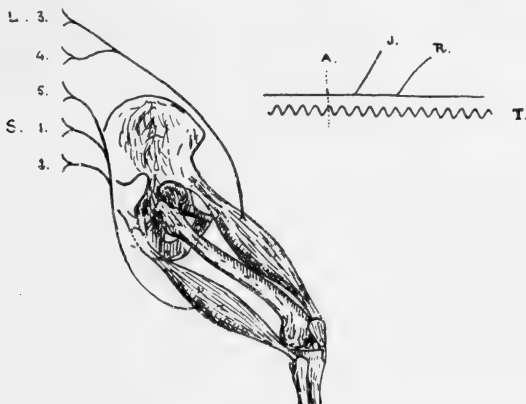


FIG. 22.—Reflex arc involved in the knee-jerk.

L., Lumbar. S., Sacral roots of the spinal nerves. To right a tracing showing short latent period of knee-jerk, J., and longer period of ordinary reflex, R.

(From Noël Paton's Essentials.)

#### 2. Pupil Reflexes.

(a) Observe the pupil of your companion while he looks (i) at a distant object, (ii) at a near object. Does any change take place in the size of the pupil ? (Accommodation Reflex.)

(b) The subject fixes his eyes upon a distant object in a good light and then closes them for a few seconds. He now opens them and again looks at the distant object. Watch the pupil carefully the moment the eyes are opened and note whether there is any change. (Light Reflex.)

Alternatively, in the optical room hold an electric lamp behind the head, then in front of the eyes, and note any change in the pupil.

(c) (i) Repeat the first experiment (a) but shade one eye with the hand and watch the pupil.

(ii) Get the subject to close one eye only, observe the other pupil now and also when the eye is opened again. Describe the changes. (Consensual Reflex.)

### 3. Skin Reflexes.

At home test the various skin reflexes described in the Text Books. (Read Reflex Action in Text Book.)

## C. STIMULATION OF NERVE

These experiments upon Reflex Action show that the nerves to the muscles are generally brought into action, *stimulated*, by changes at their origins in the spinal cord.

But they may also be stimulated at any part as is shown by I. 3, p. 31, or by the following experiment.

Press on your ulnar nerve as it passes behind the internal condyle of the humerus and note the result. To what point is the resulting sensation referred ?

*Lesson VI. To be provided for each pair of Students.*

1. Induction coil, mercury-key and wires, cells or electric supply from accumulator.—2. Frog.—3. Frog-plate on stand.—4. Pins.—5. Small glass of strong acetic acid.—6. Jelly-jar (small) filled with water.—7. Two jelly jars, one with 1 in 1000 sulphuric acid, and the other with 1 in 500 acid.—8. Blotting paper.

*For the Class.*

Microscopes with (a) a piece of nerve teased in 0.75 per cent. NaCl; (b) a transverse section of a nerve; (c) a transverse section of the spinal cord stained by Weigert-Pal's method.

## LESSON VII

### D. THE ACTION OF NERVES ON SKELETAL MUSCLE

Examine with the microscope (1) a piece of frog's muscle teased in 0.75 per cent. NaCl; (2) a preparation showing the endings of nerve in muscle.

**METHODS.**—The action of nerve on skeletal muscle may be investigated in two ways—(1) by stimulating the nerve, (2) by throwing it out of action.

Connect up an induction coil for single induced shocks, using the pin electrodes provided (see Appendix, p. 81).



1. The frog supplied has been killed by destroying its brain. A ligature has then been placed low down, round all the structures of one thigh excepting the sciatic nerve, and a dose of curare has been injected under the skin. The curare has thus acted on the nerve, but has been prevented by the ligature from reaching the gastrocnemius muscle. (Fig. 23.)

Describe the condition of this frog compared with the normal one before the cord was destroyed (p. 29, A. 1).

What has been the effect of the curare ?

Expose the sciatic nerve on the side opposite to the ligature (p. 30, 3). That on the ligatured side is already exposed. Now apply the electrodes to the structures indicated below, and stimulate by closing and opening the mercury key, noting what happens in the muscles.

(i) Limb exposed to curare (unligatured).  
 (a) Sciatic nerve ; (b) Gastrocnemius muscle.

(ii) Limb protected (ligatured). (a) Sciatic nerve (above where ligature was applied) ; (b) Gastrocnemius muscle.

Record the results on the appended table :

	Curarised.	Protected.
Nerve . . .		
Muscle . . .		

Now formulate your conclusions as to—1. What is the influence of nerve upon skeletal muscle. 2. What is the influence of curare upon (a) nerve, (b) muscle, and (c) the junction of nerve with muscle.

**Can a Muscle be Stimulated without the Intervention of the Nerve ?**

Try to answer this question from the results of the last experiment.

*E. THE STIMULATION OF NERVE AND MUSCLE BY ELECTRICITY*

**I. The Isolated Nerve-Muscle—Preparation of the Frog**

**1. Galvanic Current.**

Using the galvanic current either from the galvanic terminals of the switch-board, or from two Daniell cells in series with a rheocord in the circuit (Appendix, Fig. 77) with ordinary thick wires, *not* pin



FIG. 23.—To show the parts of the frog acted upon by curare (shaded). (From Noël Paton's Essentials.)

electrodes, study the stimulation of nerve and muscle, using the protected limb of the frog from the last experiment. Isolate the nerve to the top of the thigh and cut it across as high up as possible. Stimulate by closing and opening the mercury key, allowing a second or two to elapse between each stimulation, and increasing the strength of the current by moving the handle on the switch-board from *W* to *S*, or by moving the bridge of the rheocord. Note any difference at making and at breaking the current, taking the contraction of the muscles as an index of the stimulation of the nerve.

## 2. Do the Two Poles act in the same way?

Dip the end of the nerve furthest from the muscle momentarily into boiling water to kill it. This part will not now respond to the electric current although it will conduct it.

Place the dead bit over one wire and the nerve near the muscle over the other. Now make and then break the current (1) with the anode on the living bit of nerve, and (2) with the cathode on the living bit of nerve, and record your results on the subjoined table :

Make.		Break.	
Cath.	An.	Cath.	An.

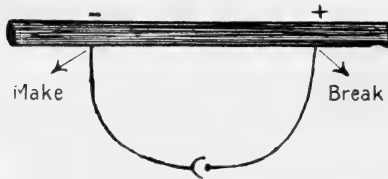


FIG. 24.—To show the stimulation at the cathode on closing and at the anode on opening in the exposed nerve.

(From Noël Paton's Essentials.)

## II. Faradic or Induced Current.

That the current instantaneously induced in the secondary coil by closing or opening the primary circuit stimulates nerve has already been learned in the curare experiment above. With the two electrodes from the secondary coil of an inductorium applied to the living bit of nerve in the preparation just used gradually weaken the induced current by moving the secondary coil away from the primary, and note that the stimulation on breaking continues after that on making has disappeared. Explain this from your knowledge of the physics of the induction coil (Fig. 25).

*Lesson VII. To be provided for each pair of Students.*

1. Frog killed, one thigh ligatured with exclusion of sciatic nerve, and curare injected.—2. Induction coil, cell, wires, electrodes and keys.—3. Three Daniell cells in series or electric supply from accumulator with resistance or rheocord to vary the strength of the current, keys and wires.

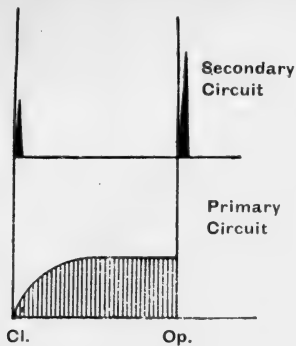


FIG. 25.—The lower line shows the current as it develops in the primary circuit (shaded part) on closing and on opening; the upper line shows the instantaneous appearance and disappearance of the current in the secondary circuit and the greater strength of the current induced on opening. In the induced current there is no separation of the make and break.

(From Noël Paton's Essentials.)

*For the Class.*

1. Microscope with piece of frog's muscle teased in 0.75 per cent. NaCl.—
2. Microscope with preparation to show the endings of nerve in muscle.

LESSON VIII

**Stimulation of Nerve and Muscle through the Skin in Man by the Galvanic Current**

Connect wires to the terminals on the switch board *G* (*galvanic current*) or lead-off from three Daniell cells in series introducing a rheocord into the circuit (p. 78). Introduce a commutator to change the direction of the current when desired (Figs. 92 and 93). Put some *saturated* salt solution in a basin and fix the end of one wire to a flat zinc electrode placed in the solution. To the end of the other wire attach a second flat zinc electrode, placing a bit of chamois leather saturated with the salt solution between the electrode and the skin. Dip the fingers of the left hand into the salt solution and apply the second electrode over the back of the thenar muscles.

First use the cathode. Beginning with a strong current, record the results which follow making and breaking the current. Then, by moving the handle on the switch-board from *S* to *W* or by means of the rheocord, reduce the strength of the current, and again record the results which follow making and breaking the current.

Now reverse the direction of the current by means of the com-

mutator,<sup>1</sup> so as to make the pole over the muscles the anode, and again study the effects of strong, medium, and weak currents at making and breaking.

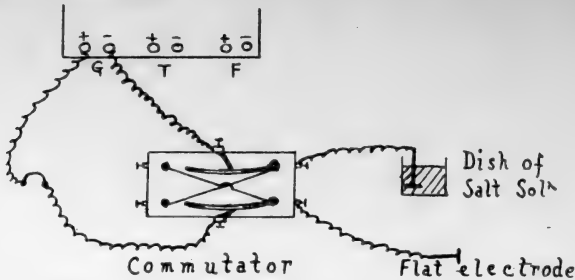


FIG. 26.—Electrical Stimulation of Muscle through Skin.

Record the results obtained on the following table :

Strength of Current.	Make.		Break.	
	Cath.	An.	Cath.	An.
Strong . . . . .				
Medium . . . . .				
Weak . . . . .				

and arrange them according to their relative effectiveness.

Compare them with the results previously obtained on the isolated nerve-muscle of the frog (p. 33, I.).

The difference between the effects of stimulation of a nerve through the skin and those which follow stimulation with the electrodes directly applied to it along its course is due to the fact that in stimulating through the skin the current passes not along but across the nerve, so that at the same point one side is under the influence of the poles placed above it and the opposite side under the influence of the other pole (Fig. 27).

(Read Electrical Stimulation of Nerve in Text Book.)

THE PASSAGE OF AN IMPULSE ALONG A NERVE

This may be investigated better after some practice in recording the contraction of muscles, and it is taken in Lesson XI.

F. WHAT HAPPENS TO A MUSCLE DURING CONTRACTION ?

Examine the biceps muscle when the forearm is flexed, and formulate the results of your observations.

<sup>1</sup> See Fig. 92, Appendix.

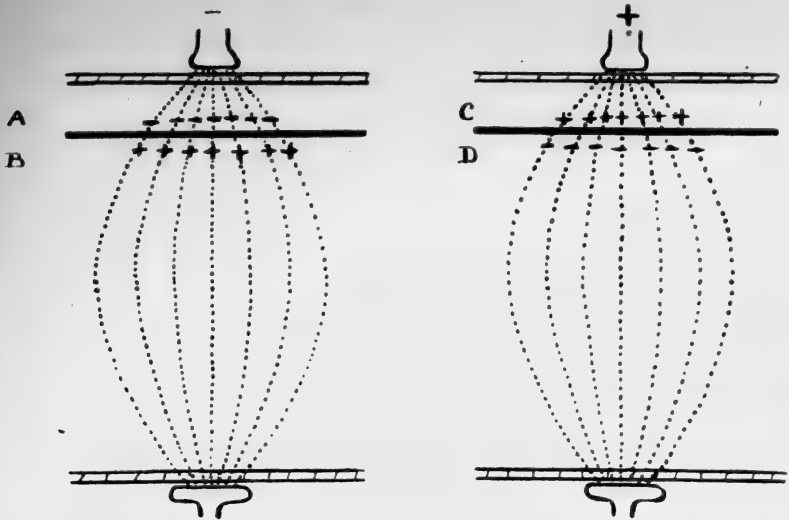


FIG. 27.—To show the stimulation of nerve or muscle under the skin at the cathode and at the anode on closing and on opening.  
(From Noël Paton's Essentials.)

**1. Extent of Contraction.**

With the hand in supination and the forearm semiflexed resting on the table measure the length of the biceps. Now flex the forearm to a small extent and measure the space through which the hand has been lifted.

From the elbow joint measure the length of the forearm and hand to the finger-tips, and also to the insertion of the biceps. Make a diagram, and from these data, and from your knowledge of the mechanism of levers, calculate the extent to which the muscle has shortened.

$$\text{Extent of contraction} = \frac{\text{length of lever from fulcrum to power}}{\text{length of lever from fulcrum to point}} \times \text{height of lift of hand.}$$

**2. Force of Contraction.**

Lift a weight of 2 kilograms in the hand, as in the previous experiment, and calculate what weight directly applied to the biceps muscle this represents.

$$= \frac{\text{length of lever from fulcrum to weight}}{\text{length of lever from fulcrum to power}} \times \text{weight lifted.}$$

This is a measure of the force of the particular contraction.

**Dynamometer.**—Study the dynamometer provided, and determine the force of contraction of the flexors of each hand.

### 3. Work Done.

From the extent of shortening of the muscle, and from the weight lifted, calculate the work done by the biceps muscle.

$$\text{Work done} = \text{weight lifted} \times \text{extent of contraction.}$$

*Lesson VIII. To be provided for each pair of Students.*

1. A galvanic current from an accumulator or from a series of cells. A resistance or rheocord. A commutator with crossed wires. Mercury key.
- 2. Wires with two flat zinc electrodes, one covered with chamois leather, and two jelly cans containing saturated NaCl solution. Dynamometer.

## LESSON IX

### The Course of Contraction of a Muscle

**METHOD.**—Make a frog's gastrocnemius muscle record its change upon a moving surface.

#### A. Apparatus.

(1) Prepare an *Induction Coil* for single induction shocks, putting the *Drum*, covered with smoked paper, in the primary circuit, so that the two strikers below the drum make and break the circuit as the drum revolves, and putting a short circuiting friction key in the secondary circuit (Fig. 28). The direction of rotation may be reversed by crossing the driving cord.

(a) Always keep the key in the primary circuit open except when using the current.

(b) Always keep the key in the secondary circuit closed except when stimulating.

(c) Keep the driving cords clear of the switch-boards.

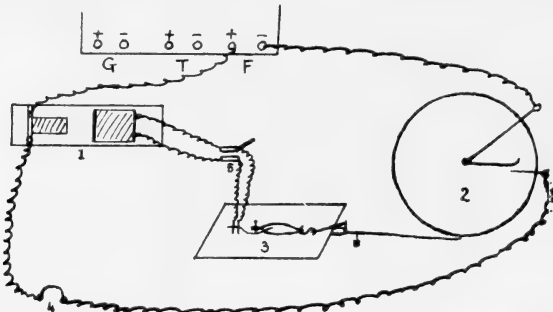


FIG. 28.—Arrangement for recording the Course of Contraction of Muscle, using switch-board instead of cells.

1. Induction Coil. 2. Drum. 3. Frog-board. 4. Mercury Key. 5. Friction Key.

(2) With the key in the secondary circuit open, move the drum with the hand in the direction in which it is driven by the string so as to make and break the circuit and test the current passing

to the electrodes by applying them to the tip of the tongue. If no current can be detected—

- (a) See that wires are not broken.
- (b) See that all metallic contacts are bright and close.
- (c) See that no short circuits are present.

(3) Arrange the driving cord of the drum to give a fairly rapid speed. (*Spindles—Large to Middle.*)

If two students are working together it is convenient to introduce two strikers under the drum in a straight line with one another, so that each revolution of the drum will give two stimulations of the muscle.

**B. Muscle-nerve Preparation.**

When everything is ready, and not before, make a nerve-muscle preparation.

(1) Kill, decapitate, and pith a frog (p. 31). Then remove the anterior part by three cuts (Fig. 29), taking care that the third cut leaves a piece of the vertebral column connected with the iliac bones. Either skin the hind legs (Fig. 29) or place the frog

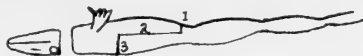


FIG. 29.—Incisions for Separation of Hind Legs.

belly downwards on a frog-board, and divide the skin at the ankle by a circular incision; expose the tendo-Achillis and pass a thread under the tendon and tie it just above the sesamoid bone. In this way a ligature is attached to the muscle without



FIG. 30. Muscles of the frog's leg. (After Ecker.)



FIG. 31.

FIG. 30.—Dorsal aspect.

- 1. Triceps femoris.
- 2. Biceps femoris.
- 3. Rectus internus.
- 4. Semi-membranosus.
- 5. Gastrocnemius.
- 6. Tendo-Achillis.

FIG. 31.—Ventral aspect.

- 1. Rectus internus.
- 2. Gracilis.
- 3. Adductor longus.
- 4. Vastus internus.
- 5. Sartorius.
- 6. Adductor brevis.
- 7. Adductor magnus.
- 8. Gastrocnemius.
- 9. Tendo-Achillis.

damage to or irritation of its fibres. The tendon is divided below the sesamoid bone, and a pull upwards towards the knee frees the gastrocnemius muscle and the skin from the remaining structures of

the leg, which are cut away just below the knee (Figs. 32 and 33). The gastrocnemius muscle is protected from drying and from contact with foreign substances by drawing down the "trouser" of skin.



FIG. 32.



FIG. 33.

Diagrams of a muscle- and nerve-preparation. (Pembrey and Phillips.)

FIG. 32.—The first stage of dissection.

FIG. 33.—The second stage of dissection. The sciatic nerve exposed and the gastrocnemius muscle covered by skin.

(2) Lay the legs down with the dorsal aspect upwards. Raise the urostyle in forceps, holding the lower end and cut up each side. Cut it across at the base, *taking care not to cut the nerves beside it.*

(3) Carefully divide the pelvis vertically in the middle line.

(4) Divide the spinal column and any soft tissues remaining in

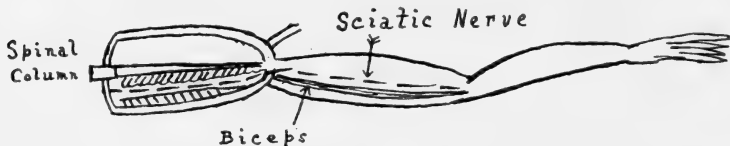


FIG. 34.—Dissection of Sciatic Nerve.

the middle line. Thus each pair of students receives one complete half. (5) Dissect out the sciatic nerve from the spinal column to the lower end of the thigh with a glass rod (Fig. 34). (6) Now separate the muscle from the tibia and cut through the fascia of the sole of the foot into which the gastrocnemius muscle is inserted, leaving a good length of it. (7) Cut away all the thigh but the lower end of the femur (Fig. 35, 1, 2, and 3), *taking care not to*



*injure the nerve.* (8) Cut through the tibia below the knee (Fig. 35, 4), and thus remove it and the foot.

C. *Bring the Preparation on to the Frog-board* (Fig. 36).

(1) Lay the muscle and nerve upon a piece of blotting paper thoroughly saturated with 0.75 per cent. NaCl solution and placed on

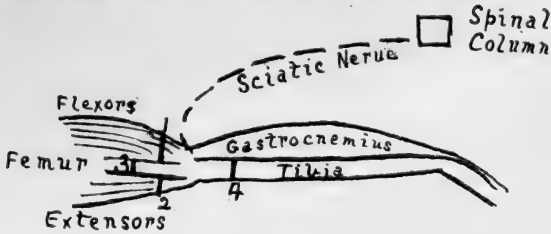


FIG. 35.

the cork plate of the frog-board. (2) Attach the tendon by a thread and hook or clip to the middle hole of the short limb of the crank lever. (3) Fix the femoral end of the muscle to the cork plate by a pin passed through the femur, so that the lever is supported in a horizontal position by the thread (Fig. 37). (4) Put a small weight, 5 grms., on the lever just clear of the stand. (5) See that the lever is not resting on the screw-pin below it. (6) Place the nerve upon the electrodes, which may be fixed to the cork by a pin. (7) Make and break the current by moving the drum with the hand *in one direction* so as not to displace the striker, and move the secondary coil out till breaking alone causes a contraction.



FIG. 36.—Myograph stand with crank lever.

D. *Take Trace.*

(1) Now bring the point of the lever lightly against the smoked surface of the drum, (a) pointing it in the direction in which the drum travels, (b) and taking care that the movable base-piece of the frog-board is pushed thoroughly home, so that the lever may be swung off and replaced with exactly the same pressure on the drum when required. (2) With the finger raise the lever to see that it marks properly, and if necessary adjust the lever by twisting the attachment so that it moves in the vertical plane. (3) With the key in the secondary circuit closed start the drum, and, when it is revolving steadily,

open the key in the secondary circuit so that the current may reach the electrodes and nerve. When the muscle is stimulated it contracts and pulls up the lever and records the contraction on the drum.

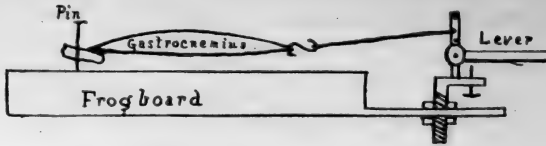


FIG. 37.—Muscle attached to Lever.

(4) Whenever the two records are made, close the key again and stop the drum. (Don't reverse the drum and don't move the stand of the frog-board.)

E. *Mark the Moment of Stimulation.*

To mark the moment of stimulation, revolve the drum slowly with the hand, keeping the key in the secondary shut till the striker is just about to make contact, then open the key and very cautiously continue the movement of the drum till the muscle contracts and marks the moment of stimulation. Note the relationship of this mark, which indicates the moment of stimulation, to the upstroke which marks the contraction.

F. *Make a Time Trace.*

(1) Swing the lever off the paper by using the basepiece (do not move the drum or the myograph stand). (2) Run off a time trace in  $\frac{1}{100}$  sec. with the tuning fork (p. 28).

G. *Record the Nature of the Experiment.*

With a pin or other sharp-pointed instrument write upon the paper the nature of the experiment, your name and the date.

H. *Fix the Trace.*

Remove the paper from the drum, and fix the trace by passing it through photographic varnish. Hang it up to dry.

I. When dry read the trace, and work out the results—

(a) **The Duration of**—1st. The time between the application of the stimulation and the contraction.

2nd. The time taken up by the contraction.

3rd. The time of relaxation.

(b) **Extent of Contraction.** Measure the extent of movement and the length of each limb of the lever, and calculate the actual shortening of the muscle as in 1, p. 37.

Measure the length of the muscle, and calculate the percentage shortening.

(c) **Weight Lifted.** Measure the distance of the weight from the fulcrum, and calculate the actual weight lifted by the muscle as in 2, p. 37.

(d) **Work Done.** Calculate the work done by the muscle as in 3, p. 38.

*Preserve the trace and the calculations in your note-book as usual.*

*Lesson IX. To be provided for each pair of Students.*

1. Induction coil, keys, wires and pair of electrodes.—2. Bottle of 0.75 NaCl solution.—3. Strips of blotting paper.—4. Myograph stand and lever.—5. Drum.—6. Tuning fork beating 100 times per second with marking wire attached.—7. Frog.

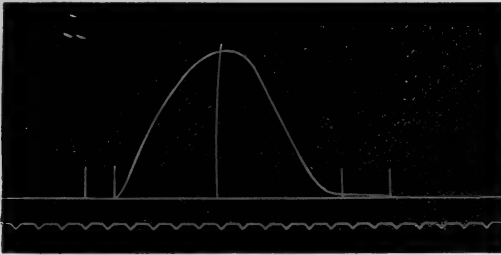


FIG. 38.—Single contraction of gastrocnemius in response to a maximal make shock.

Muscle loaded with lever and 30 grms. at axis of lever; actual load on muscle, 6 grms. Magnification, 5. Temp., 15° C. Time marker, 100 per sec. (A.P.B.)

LESSON X

G. INFLUENCE OF VARIOUS FACTORS UPON MUSCULAR CONTRACTION

Arrange an experiment in the same way as the last, and then proceed as described in 1, 2, 3, 4 and 5. In each experiment mark the point of stimulation.

*Each pair of students does one or more of the following experiments, and then compares their tracings with those of others doing the other experiments.*

**1. The Effect of the Length of the Muscle on the Contraction and Work Done.**

A. With the screw-pin below the lever well depressed, attach a weight to the lever such that the muscle in contracting raises it to near the maximum (about 10 grms.), and with the muscle thus lengthened by the weight record a contraction.

B. Now screw up the pin below the lever so as just to prevent the weight stretching the muscle. Adjust the level of the drum so that the lever marks on the same base line as before, and again take a trace over the last on the drum.

Mark the moment of stimulation, etc., as before. Fix and compare the two traces, and work out the extent of contraction and the work done in each.

**2. Influence of Load.**

1. *On the course of Contraction.* METHOD.—(1) Take a trace of a muscle twitch in the usual way but with no weight on the lever.

(2) Closing the key in the secondary circuit when the trace is made, stop the drum and swing the lever off. (3) Hang a weight of 10 grms. on the lever so that the thread from the muscle and that from the weight are equidistant from the fulcrum, or each at a measured distance from the fulcrum. (4) See that the lever is not resting on the screw pin. (5) Lower the drum till the point of the lever

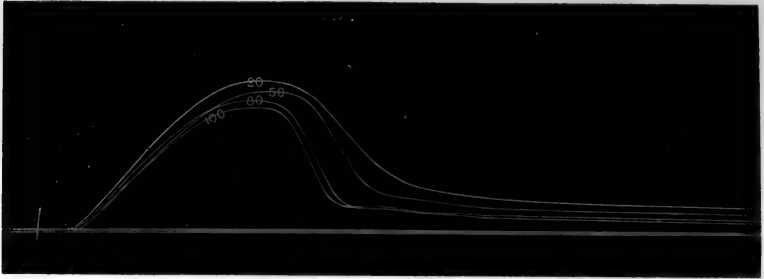


FIG. 39.—The effect of load upon the contraction of the gastrocnemius muscle. (A.P.B.)

marks the same abscissa as before and take another trace when the drum is running uniformly. (6) Repeat the experiment till all the weights of the series supplied have been used. If the lever is much depressed, shift the pin holding the muscle to make it again horizontal; *but, in doing so, do not move the stand.* Number the

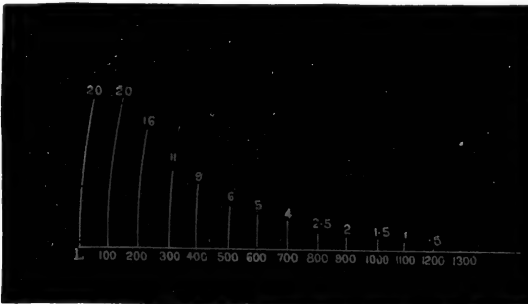


FIG. 40.—Height of contractions of gastrocnemius with increasing load.

The number above each contraction is its observed height in mm. Magnification, 5. The number below each contraction is the weight in grm. hung at the axis of the lever; the actual load on the muscle was half of this number. (A.P.B.)

traces, and, having calculated the actual weight applied to the muscle (p. 42, I. c.), note it upon the drum.

Mark the point of stimulation, take a time trace and fix.

2. *On the Extent of Contraction.* METHOD.—Proceed as in p. 46, 4, 2, but instead of varying the strength of the stimulus go on increasing the weight attached to the lever, when necessary adjusting

the level of the drum, and mark under each upstroke the weight used, calculated as directly applied to the muscle.

Make a diagram, showing and comparing the work done with each weight.

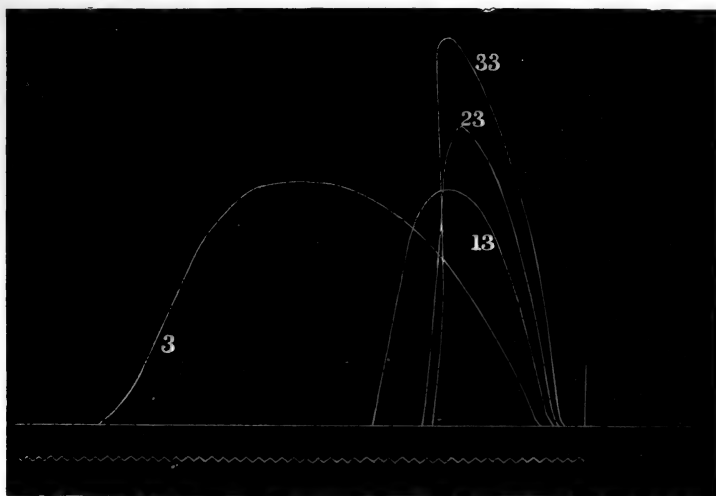


FIG. 41.—The effect of temperature upon the contraction of the gastrocnemius muscle.

The time is marked in  $\frac{1}{100}$  second. The tracing should be read from right to left. Figures on curve are the temperatures of the salt solution. (Pembrey and Phillips.)

### 3. Effect of Varying the Temperature.

METHOD.—After recording a normal twitch, swing the lever off the drum by the base-piece, and cool down the muscle by putting

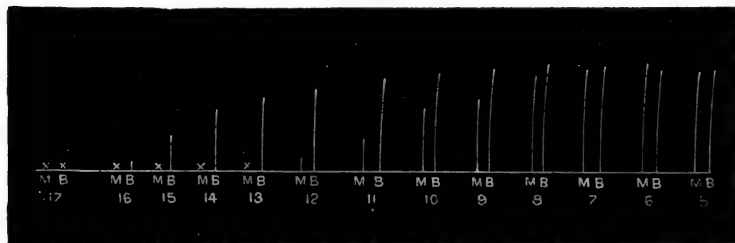


FIG. 42.—Heights of contraction of a muscle with different strengths of stimuli. M marks the make and B the break of the primary circuit. The numbers refer to the distances in cms. of the secondary from the primary coil. (A.P.B.)

ice round it, separating the ice from the muscle by a piece of blotting paper saturated with normal saline. After 2 or 3 minutes remove the ice and paper and swing the lever on to the previous abscissal line, raising or lowering the drum if this is necessary, and when the

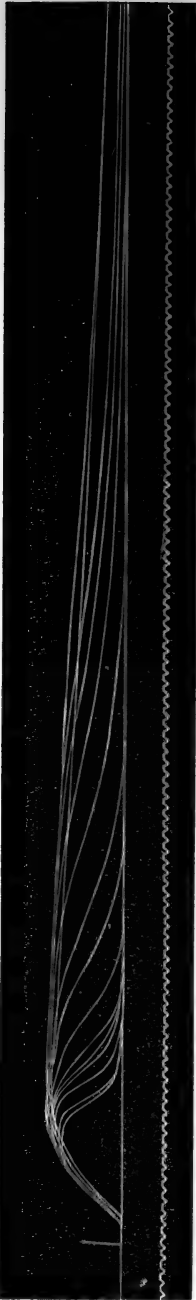


FIG. 43.—Series of contractions of gastrocnemius to show the change in contraction as the muscle becomes fatigued. Every twentieth contraction recorded. Actual load on muscle, 10 grms. Magnification, 5. Temp., 12° C. Time tracing, 100 per sec. (A.P.B.)

drum is again running at uniform speed take another trace *over the first*.

Again swing the lever off the drum by means of the base-piece and then, proceeding in the same way, warm the muscle by allowing normal saline at 25° C. to run over it for 2 or 3 minutes, and take another tracing. (*Put a plate under the frog-board to catch the solution.*)

Number the curves, and note "normal," "cold," and "warm" upon them.

Take a time trace, fix, and work out as on p. 41, I. (a), (b), (c) and (d).

#### 4. Effect of Varying the Strength of the Stimulus.

##### 1. On the Course of Contraction.

METHOD.—Starting with the lever off the drum find the smallest stimulus which will give a contraction, i.e. find the furthest point of the secondary coil from the primary at which a contraction can be got. Then take a tracing as described above and note the position of the secondary coil. Now push the secondary coil nearer the primary, *note its distance*, and take a second record when the drum is running at a uniform speed. Repeat this, each time moving the secondary coil nearer the primary. Number the curves and write upon the drum the distance of the secondary coil from the primary in each. Mark the point of stimulation, take a time tracing and fix. Study the effect of varying the strength of the stimulus on (1) the duration of the phases and (2) the extent of contraction.

If, with the strongest stimulus used, a shoulder should appear on the ascent of the curve, consider how it has been caused.

##### 2. On the Extent of Contraction.—

(1) Disconnect the drum from the primary circuit, twist the ends of the wires together, and use the mercury key to make and break the current. (2) Bring the lever against the drum unconnected with the driving wheel

and, with the drum stationary, record the contraction of the muscle unloaded, then moving the drum about a quarter of an inch between each record, and keeping the make and the break upstrokes separate, the effect of the minimal effective stimulus (make and break), and of stronger and stronger stimuli.

Mark under each upstroke the distance of the secondary coil from the primary, fix, and work out as on p. 42, I.

### 5. Effect of Continued Exercise.

(This should be done after 1, 2, 3, or 4 on the same preparation.)

**METHOD.**—Having arranged the apparatus for taking a trace of a muscle contraction, start the drum and let the muscle be stimulated, and record its contraction with each tenth revolution of the drum. To do this, after a contraction or two contractions are recorded, swing the lever off the paper by the base-piece of the stand; let the muscle make nine contractions, then swing the point of the lever on and record the next two contractions. Repeat this process as long as the muscle contracts. Number the curves and

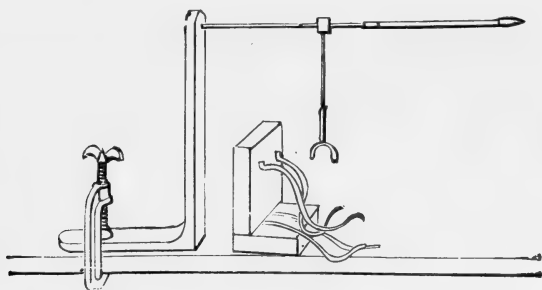


FIG. 44.—Spring Ergograph. (Porter.)

write on the drum the number of stimuli which have preceded each. In this way study the effect of continued exercise on muscular contraction.

Take a time tracing, fix, and work out as on p. 42, I.

*After taking each trace formulate your conclusions as to the effect of the particular condition.*

**The Result of Continued Exercise on the Neuro-muscular Mechanism in Man.**—**METHOD.**—Fit the hand and arm in a Mosso's ergograph, to the hook of which a weight of 3 kilograms has been attached, or use Porter's ergograph (Fig. 44). Bring the writing point against a *very* slowly moving drum. Set a metronome beating about sixty times per minute, and as each beat is heard raise and lower the weight with the finger to the fullest extent as long as it is possible to move the weight, then study the record of the onset of fatigue upon the drum. Note the time taken by your watch. Compare your record with those of others,

The trace upon the drum must not be looked at during the experiment.

(Read Simple Muscular Contraction and factors modifying it in Text Book.)

*Lesson X. Apparatus as in last lesson.*

1. Series of weights to attach to lever 5-10-20-30 gms.—2. Normal saline.
- 3. Broken ice.

*To be provided for Class.*

Mosso's or Porter's Ergograph.

## LESSON XI

### 6. The Effect of a Rapid Succession of Stimuli.

**METHODS.**—1. With a fast drum, arrange the two strikers on the axle, well apart from one another, and, with breaking shocks, take a trace of two muscle twitches near the bottom of the drum. Mark the moments of stimulation. Then approximate the strikers so that the second contraction will be produced before the first has

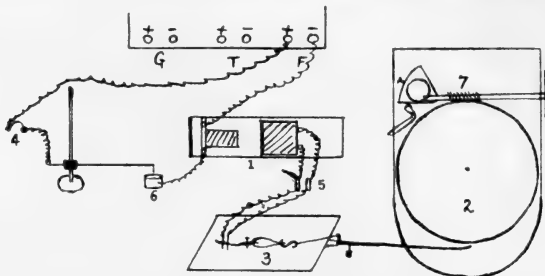


FIG. 45.—Arrangement for investigating the Effect of a Rapid Succession of Stimuli.

- |                    |  |
|--------------------|--|
| 1. Induction Coil. | 5. Friction Key.                                 |
| 2. Drum.           | 6. Vibrating Spring with Cup containing Mercury. |
| 3. Frog-board.     | 7. Gear for producing Slow Rotation of the Drum. |
| 4. Mercury Key.    |  |

ceased, move the drum so that the lever writes at a higher level, and take a trace.

Note the relationship of the second contraction to the first, upon which it is superimposed. Mark the moments of stimulation, and take a time trace.

2. Now disconnect the drum from the primary circuit and introduce a spring (Fig. 45 and Appendix or as in Fig. 47). Adjust the current to stimulate on breaking. (1) Set the drum going *slowly* (*slow gear—large spindle to small*), and set the spring so that it makes and breaks three to five times per second (timing with your watch), and note its length. The arrangement shown in Fig. 47 may be used. Bring the point of the lever upon the drum. Start the spring vibrating. Open the key in the secondary circuit



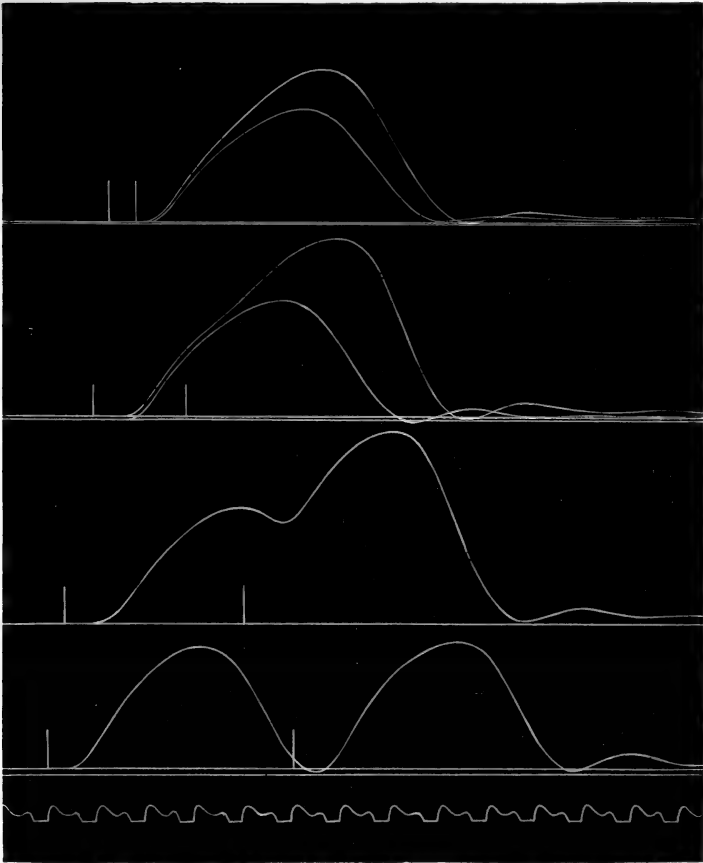


FIG. 46.—Effect of two successive maximal stimuli, with gradually diminishing intervals, upon the gastrocnemius. To be read from below upwards.

Time tracing, 50 per sec. In the two upper curves are shown both the contraction in response to the first stimulus alone and the combined contractions caused by the two successive stimuli. (M.S.P.)

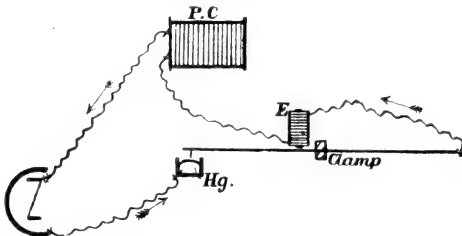


FIG. 47.—Diagram of the vibrating reed in circuit.

and take a trace for about 3 or 4 seconds, then close the key, *but let the drum run till the lever falls to its previous level, then stop it.*

(2) Now move the lever to another part of the drum, shorten the spring, and take another trace of the same duration.

(3) Repeat several times, shortening the spring and each time noting its length.

(4) Finally connect up the Neef's hammer of the induction coil—a very short rapidly-vibrating spring (p. 83) and take another tracing. Take a time trace of intervals of  $\frac{1}{10}$  sec. by means of an electromagnetic marker. Fix the tracings and study the results of a succession of stimuli, and formulate your conclusions from these results.

(Read Physiological Tetanus in Text Book.)

#### H. CONTRACTION OF HUMAN MUSCLE.

If frogs are not available, human muscle may be used by the following method.

The wires from the short-circuiting key in the secondary circuit (Fig. 28, 5) are fitted with electrodes similar to those used for stimulating nerve and muscle through the skin (p. 35,) and covered with chamois leather. Before using the electrodes they are soaked in a saturated solution of common salt.

The angled lever of the frog-board has attached to the highest hole in the vertical limit a thread rather longer than the frog-board with a slip noose at the end.

The recording limb of the lever should be short and a weight of 40 or 50 grms. should be attached to it.

A wooden platform, readily made from an old box, is placed on the table behind the frog-board and the top should be at the height above the ground of the subject's elbow when standing beside the table.

The forearm is bared and its dorsal aspect rested upon the platform with the fingers in the natural position of semi-flexion. A piece of tape fixes the first phalanges to the board and another piece is placed round the forearm to steady it. The noose is now placed round the terminal phalanx of the middle or ring finger, and by altering the position of the arm or the platform the thread is tightened till the recording limb of the lever is horizontal.

The skin below the elbow is well moistened with salt solution and the subject holds the flat electrode upon it.

The experimenter finds the motor point for the flexor sublimis digitorum acting upon the finger used and marks it with a skin-pencil so that the electrode may, each time it is used, be applied at exactly the same spot and with the same pressure.

Having adjusted the secondary coil so that a good contraction to breaking the primary circuit is obtained, the point of the lever is swung against the drum. The drum is started and when revolving uniformly the short circuiting key in the secondary is opened and a contraction recorded. The moment of stimulation is marked in

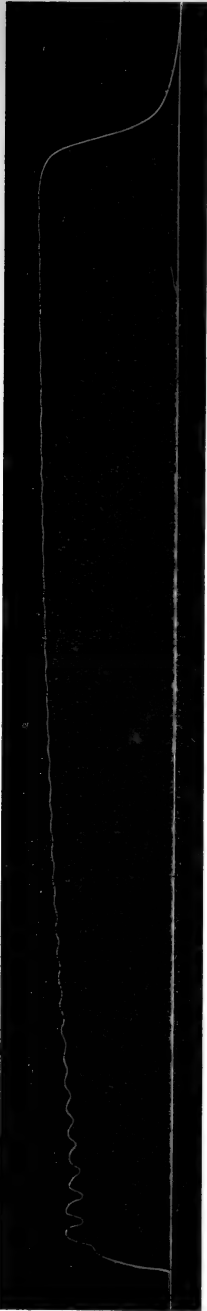


FIG. 48.—Incomplete tetanus of gastrocnemius.  
20 stimuli per sec. Temp., 15° C. (M.S.P.)

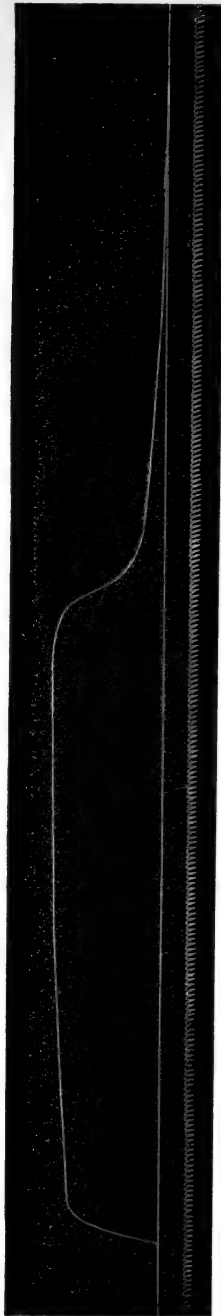


FIG. 49.—Same preparation as Fig. 48.  
30 stimuli per sec. Time marker, 50 per sec. Temp., 15° C. (M.S.P.)

the way described on p. 42, E, and a time tracing in  $\frac{1}{100}$ th seconds is recorded under the trace as described on p. 42, F.

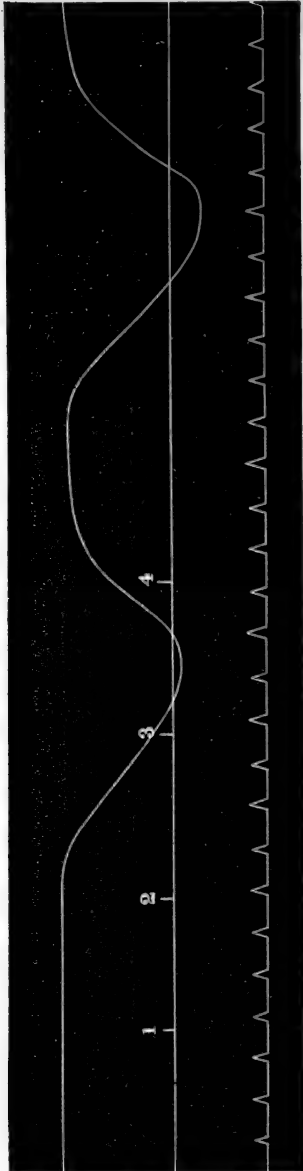


FIG. 50.—Record of contractions of a Stanniused Ventricle. Excitations were given at the points marked 1, 2, 3, and 4. The second and third stimuli during systole were ineffectual. The fourth stimulus excited a second contraction. The contraction is represented by the down stroke. The time is marked in fifths of a second. (L.H.)

The effect of varying the *strength of the stimulus* may be investigated as on p. 46.

The influence of *load* may be studied by placing a rubber band round the recording arm of the lever and the end of the frog-board and increasing the load by moving the band outwards on the lever (p. 43).

The influence of *successive stimuli* and the *genesis of tetanus* may also be studied (p. 48, Lesson XI. 2).

## J. THE CONTRACTION OF CARDIAC AND OF VISCERAL MUSCLE

### 1. Character of a Single Ventricular Contraction (Cardiac Muscle).

Use the body of the frog from which you made your muscle nerve preparation. Expose the heart as described in Lesson XIV. p. 61, and separate the sinus from the auricles by applying the first Stannius ligature (p. 64).

After the ventricle has been stopped take a trace on a moderately fast drum (*fast-gear*—*small spindle to the largest on the drum*) of the contraction caused by touching the ventricle. The touch will record the moment of stimulation on the drum. Take a time trace in  $\frac{1}{10}$ th sec. and measure the duration of the phases.

### 2. Character of the Contractions of Muscle of Frog's Stomach (Visceral Muscle).

Kill a frog and open the abdomen. Identify the stomach and cut with sharp scissors a ring about two or three millimetres wide

from the cardiac end or from the œsophagus. Fix this to the frog-board by means of a bent pin passed through the lumen. Connect it with a heart lever by a thread and hook (Fig. 59). Make the lever write on a *very slow* drum. Keep the preparation moist and observe whether any movements are recorded. Pinch the ring with forceps and record the resulting contraction.

The action of certain drugs may be studied on this preparation.

Under the microscope examine teased preparations of skeletal, cardiac and visceral muscle fibres and a preparation of the endings of motor nerves in skeletal muscles.

K. THE PASSAGE OF AN IMPULSE ALONG A NERVE

(This belongs to Lesson VIII.)

METHOD.—Place a commutator *with the cross wires removed* in the secondary circuit of an induction coil, and connect a pair of pin electrodes with each pair of the terminals, so that the current may be sent into one or other of the pairs of electrodes (see p. 84). Make a nerve-muscle preparation with the nerve complete to the spinal column, arrange the preparation on the frog-board and place the nerve upon the electrodes—one pair near to and one pair *as far as possible* from the muscle. Bring the lever against a *very fast* drum, and take a separate tracing of the muscle twitch with the

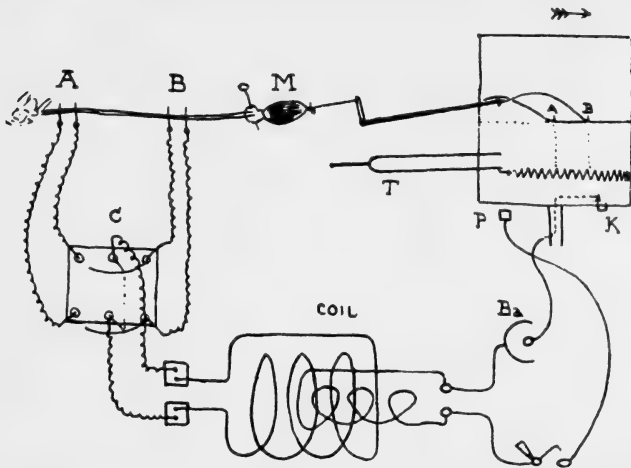


FIG. 51.—Arrangement for investigating the rate of passage of an impulse along a nerve.

nerve stimulated through each pair of electrodes. Finally, put a time tracing of  $\frac{1}{100}$  of a second on the drum and measure the length of nerve between the two pairs of electrodes (Fig. 51).

Lesson XI. Apparatus as in last lessons.

Tetanus Spring. Microscopes with specimens of teased cardiac and visceral muscle.

## SECTION III

# CIRCULATION

### LESSON XII

#### HEART

##### I. Structure.

(Drawings should be made of the various structures and a Text Book of Anatomy may be consulted.)

1. Use the sheep's heart supplied. Open the right auricle by a horizontal cut. Open the right ventricle either by an inverted V incision as demonstrated or by passing scissors down the pulmonary artery into the ventricle and snipping through the anterior wall.

Examine the tricuspid valve and papillary muscles.

Slit up the pulmonary artery and examine the semilunar valves.

Open the left auricle and ventricle by a vertical antero-posterior incision through the left auriculo-ventricular orifice and middle of the aorta. This is best done by laying the heart on its anterior surface and cutting from behind. Examine the mitral valve and papillary muscles, the relations of the anterior cusp of the mitral to the posterior aortic wall and the aortic semilunar valves and mouths of the coronary arteries.

On the septum between the ventricles note that a special band of muscular fibres passes from the auricles to the ventricles.

2. On the models of the thoracic organs, study the attachments and relations of the heart to the anterior and posterior chest walls, to the central tendon of the diaphragm and to the lungs. Note that the lungs, heart and great vessels completely fill the thorax.

3. In a boiled heart of a sheep, twist off the auricles, aorta and pulmonary artery, and examine the auriculo-ventricular and pulmonary fibrous rings from which the muscular fibres rise.

Clear the visceral pericardium off the ventricles and study the course of the muscle fibres.

4. In the longitudinal section of the heart of a mouse under a low power of the microscope, study the various parts.

5. Dissect the heart of a dead frog. Identify the sinus, auricles, ventricle and bulbus arteriosus (Figs. 52 and 61).

Thrust a small test-tube down the gullet to stretch it, and dissect out the vago-sympathetic nerve and follow its cardiac branch down to the heart.

The inspection of the frog's heart in action (p. 61, Lesson XIV. I.) may with advantage be taken here if the supply of frogs is sufficient.



FIG. 52.—Heart of frog from behind.

- |               |                 |
|---------------|-----------------|
| 1. Ventricle. | 4. Sinus.       |
| 2. Auricle.   | 5. Great Veins. |
| 3. Crescent.  | 6. Aorta.       |

## II. The External Manifestations of the Cardiac Cycle in Man

### 1. The Cardiac Impulse—Cardiograph.

Get ready and study the mode of action of a cardiograph (Fig. 53), which should have a long lever on the recording tambour.

(1) Find the position of the cardiac impulse on the front of the chest of a fellow student and investigate its characters.

(2) Mark its position with an aniline pencil and then apply the cardiograph with the button upon the impulse. Adjust the pressure

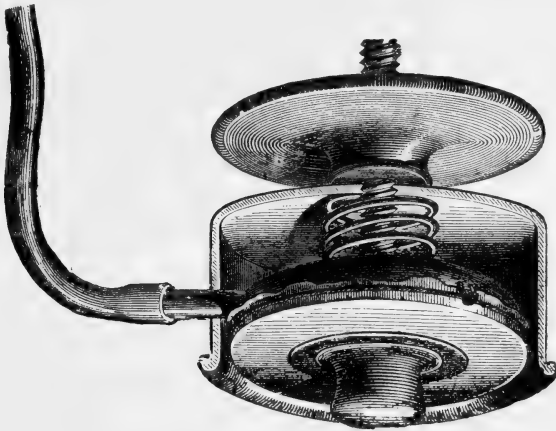


FIG. 53.—Marey's cardiograph.

The tube is connected with a recording tambour. Pressure is adjusted by the screw and spring.

of the button by means of the screw till the lever gives the largest possible excursion, and take a tracing on a slow-moving drum. This is best done with the subject seated and leaning forward and to the left. The breath may be held in expiration for a few seconds. Take a time tracing in  $\frac{1}{10}$  seconds. Make an enlarged drawing of a part of the trace and try to explain the various elevations and depressions.

## 2. Sounds of the Heart.

With the stethoscope provided, listen over the cardiac impulse and over the second right costal cartilage. Put a finger on the cardiac impulse and try to time the sounds heard in relationship to this. Note the characters of the sounds.

## 3. The Arterial Pulse (see p. 57).

### III. Cardio-pneumatic Movements. Do the Movements of the Heart cause Movements of the Air in the Air Passages?

Fill the mouth, nose and pharynx with tobacco or other smoke. Hold the nostrils. Insert in the mouth the wide end of a wide bore glass tube drawn to a somewhat fine point. Stop breathing and keep the glottis open. Note any movement of the smoke in the tube, and time it with the cardiac impulse.

What conclusion do you draw as to the influence of the cardiac cycle upon the movements of air in the air passages?

*Lesson XII. To be provided for each pair of Students.*

1. Sheep's heart, fresh or preserved in formalin.—2. Dead frog, preserved in formalin.—3. Cardiograph and recording drum.—4. Stethoscope.—5. Long wide bore glass tube, drawn to fine opening.

*For the Class.*

Microscope with a low power and a specimen of a longitudinal section of the heart of a mouse. Models of sections of the thorax.

## LESSON XIII

### CIRCULATION IN THE BLOOD VESSELS.

Examine the circulation in the web of the foot of a frog or in a tadpole's tail under the microscope. Identify arterioles, capillaries and veins and notice the character of the flow in each.

#### I. Schema of Circulation.

##### 1. General Distribution of Pressure.

Examine the schema of the blood vessels made of elastic tubes provided, and identify the parts representing arteries, capillaries and veins. Attach the arterial end to the water tap or to the nozzle of a Higginson's syringe in a basin of water and fix vertically in stands the two glass tubes connected with the arteries and veins respectively. Cautiously turn on the water or gently pump by means of the syringe and measure the pressure in the arteries and in the veins, and calculate it in mm. of mercury. Note the effect of (a) varying the force of inflow by turning off and on the tap or by varying the force of the pumping, (b) varying the resistance to outflow by constricting the arterial tubes.



## 2. The Arterial Pulse.

(a) With the finger, compress and relax the arterial tube near the tap at regular rhythmic intervals of about a second, so as to imitate the interrupted inflow of blood from the heart or compress the syringe rhythmically at this rate. Note the effect of this upon the arterial and venous pressures, and study the further effect of constricting the arterial tubes near the capillaries upon the movements of each of the columns of fluid.

(b) Place a finger on the arterial tube and note the expansion, the *pulse*, with each rhythmic inflow, and repeat the observation on the venous tubes. Explain any difference which may be observed.

## II. The Pulse in Man.

### 1. Relation to the Cardiac Impulse.

(1) Place a finger of the left hand on the radial artery at the right wrist while feeling the cardiac impulse with the right hand, and note what is felt in the artery. Determine whether the change is simultaneous with the cardiac impulse.

(2) Does the wave develop simultaneously throughout the arterial system or does it pass out to the periphery? Place one finger over the carotid and another over the radial artery and time the appearance of the wave under each.

(3) Place the finger on the radial artery of a companion and study the pulse as to (a) rate, (b) rhythm, (c) tension, (d) volume, (e) form of wave.

(1) *Rate*.—Count the pulse for half a minute. This gives the rate of the heart beat. (2) *Rhythm*.—Note whether the pulse is regular or irregular as to the *rate* and as to the *strength* of the individual beats. This indicates regularity or irregularity in rate and strength of the ventricular contractions. (3) *Volume*.—A big pulse means a marked difference between the maximum and minimum pressures in the artery. (4) *Tension* or force of the pulse wave (maximum systolic pressure). This may be estimated by placing the middle finger on the radial pulse, laying the forefinger on the artery just above it and pressing on the vessel till the pulse is no longer felt under the middle finger. If a recurrent pulse comes through the palmar arch this may be obliterated by pressure with the ring finger distally to the middle finger. (5) *Form of the Pulse Wave*.—Does the wave come up suddenly and disappear suddenly or gradually? Can more than one crest be felt on the wave? If a second crest can be felt it is the dicrotic crest and the pulse is said to be dicrotic.

(4) **The Sphygmograph.** Using Dudgeon's or Marey's sphygmograph, under the direction of the demonstrator, take a tracing of the radial pulse. (i) See that the clockwork of the instrument is wound up. (ii) Feel the radial pulse (*note its rate*). (iii) Mark the artery with an aniline pencil. (iv) Rest the back of the wrist upon a book or other support with the hand slightly dorsi-flexed.

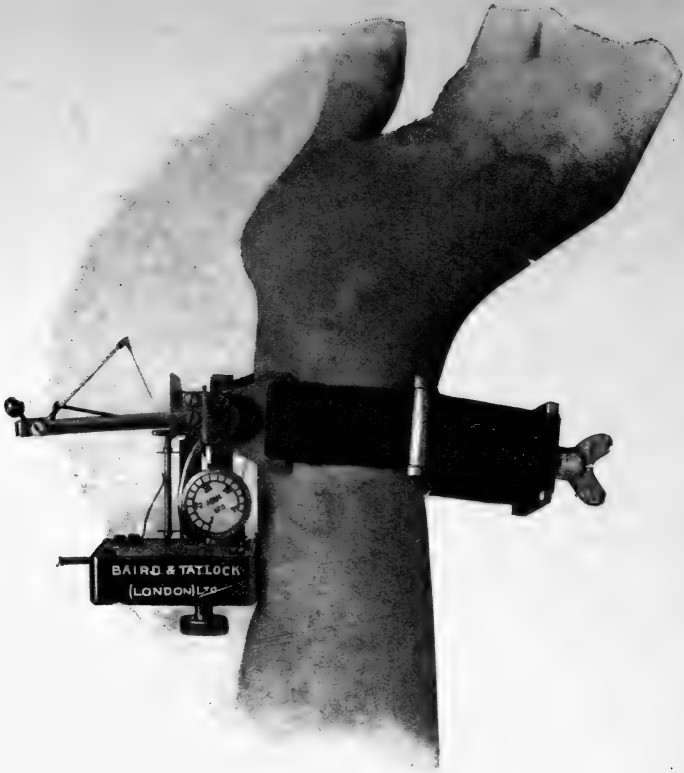


FIG. 54.—Dudgeon's sphygmograph.

(v) See that the button on the spring of the sphygmograph is over the artery. (vi) Strap the instrument on, varying the tightness of the band to give the best range of movement. (vii) Vary the



FIG. 55.—Marey's sphygmograph.

tension of the spring by means of the eccentric till the largest range of movement of the lever is secured. (viii) Slip the smoked paper under the wheels and under the point of the lever, using a pin to raise the point of the latter if necessary. (ix) Now start the clock-work and run off a tracing. (x) Mark upon it the name and age of the subject, the date and the rate of the heart per minute, and

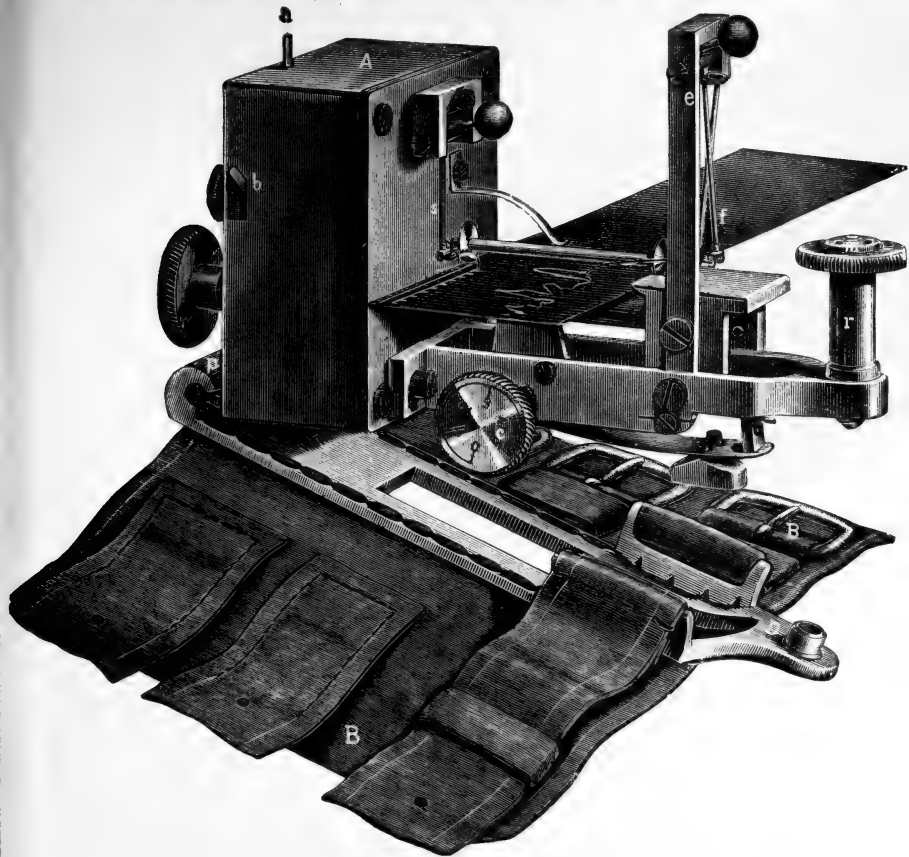


FIG. 56.—Sphygmograph provided with time writer (Jacquet)

fix it. Copy it carefully, and try to explain the various elevations and depressions with reference to the events in the cardiac cycle as shown by the cardiograph (p. 55).

(Read the Arterial Pulse in a Text Book.)

### III. Blood Pressure in Man

Make an observation of the arterial blood pressure of a companion by the **Riva Rocci Instrument**.

(a) Examine the manometer and see that, if there is a cap, it is removed and that the mercury is at zero.

(b) Enclose the upper arm of the subject in the armlet and, with the arm horizontal and at the level of the heart, place one finger on the radial pulse and raise the pressure in the instrument some 3 cm. above the point at which the pulse disappears. (*Take care not to pull on the tube.*)

(c) Release the pressure gradually and note carefully the exact height of the mercury at the moment when the pulse returns at the wrist. This is the *maximum systolic pressure*.

Repeat the observation, but instead of feeling the pulse listen over the artery near the elbow with a binaural stethoscope, and note the pressure at which a sound is first heard (*maximum systolic pressure*), and go on slowly releasing till the sound disappears (*diastolic pressure*).

It is obvious that if the pressure under the armlet is just sufficient

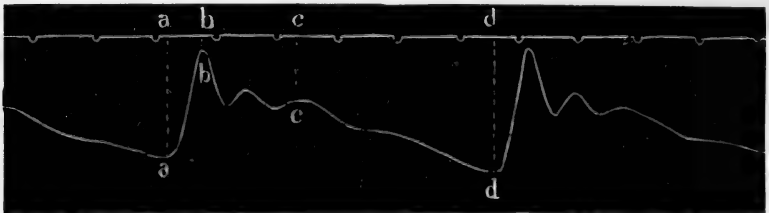


FIG. 57.—Pulse tracing (sphygmogram) taken by Jacquet's sphygmograph.  
*a d* = the period of the pulse curve, *b* = the primary, *c* = the diastolic wave.  
 Time marked in fifths of a second.

to prevent the arterial pulse, i.e. the maximum systolic pressure, from passing that it must give a measure of this systolic pressure.

The lowest pressure in the artery, the diastolic pressure, which occurs between the heart beats, is given by the disappearance of the murmur or whiff-like sound, because this is caused by the constriction of the artery by the armlet when the pressure in it is sufficient to compress the artery, but at the point when it becomes insufficient the murmur disappears.

(Read Arterial Blood Pressure in Text Book.)

#### IV. Effect of Mental and Muscular Work on the Circulation and Respiration

(*This Experiment is better done after Respiration, p. 73.*)

A. 1. Have ready the apparatus to record the respiration from the nostril of a fellow student (p. 72, 6).

2. Place the bag of the Riva Rocci apparatus round his arm.

3. The subject holds the other hand out from the side and raises and lowers it as directed. The observer determines at what level of the hand the veins disappear while the subject is sitting still.

4. Note the colour of the face.

**B.** Having recorded (1) the respiration and (2) the systolic blood pressure, (3) counted the pulse and (4) measured the height of the hand above the heart at which the veins collapse, make the subject perform 10 minutes' strenuous mental work and again examine and record systolic arterial blood pressure, pulse, venous pressure and respiration, and note the colour of the face.

**C.** Now make him take strenuous muscular exercise till he is breathless, and repeat all the observations.

Formulate your conclusions as regards the effect of mental work and of muscular work upon—

The rate of the heart.

The systolic arterial pressure.

The condition of the peripheral vessels.

The venous pressure.

The rate and depth of respiration.

*Lesson XIII. To be provided for each pair of Students.*

1. Schema of circulation.—2. Web of frog's foot under low power of microscope.—3. Dudgeon's sphygmograph.—4. Riva Rocci apparatus.—5. Stethoscope (binaural).—6. Recording tambour and tube.

## LESSON XIV

### MODE OF ACTION OF THE HEART IN THE FROG

#### I. The Cardiac Cycle

##### 1. Study the Exposed Heart in the Frog.

Kill a frog by cutting off its head *behind the tympanic membranes* or by thrusting a stout pin into the occipito-atlantoid joint and destroying the brain. Thrust a thick pin down the spinal canal (see Fig. 21, p. 31).

Pin the body on its back on a cork plate. Open the abdomen by an incision in the middle line, and carry the incision up through the shoulder girdle in the *middle line*, taking care that the point of the scissors does not injure the heart. Separate widely the two sides of the girdle, pinning each back *firmly*, and thus expose the heart in the pericardium. Snip through the pericardium and study the auricles, ventricle and bulbus as seen from the front.

Study and describe the changes in shape which each part undergoes—the relative duration of each change in each part and the sequence of events in the different parts—and record your observations. Time and note the number of contractions of the ventricle in one minute.

Now take the *tip* of the ventricle in fine-pointed forceps or pass a fine needle with a fine thread through the tip of the ventricle, lift it up and observe a fold of pericardium, the frænum, which is attached to it behind, and carefully snip this through. Then turn the ventricle freely forward and study the changes which occur in the sinus venosus, and the relation of these changes to the changes in the other parts of the heart.

## 2. To Record the Cardiac Cycle.

Place the cork plate with the frog directly under the heart lever, which should have an angled swinging glass point to decrease friction. Pin the frog *firmly* on the board by two pins *close to the heart*, but not through the vagus nerve, fix the legs also by pins and attach the lever to the *apex* by means of a small clip, or use the thread already inserted, *seeing that the thread is vertical* (Fig. 59). Adjust the lever (*a*) by its height on the stand (*b*) by the spiral spring, so that each heart beat causes the largest range of movement. Then bring the glass point of the lever *lightly* against the drum; start the drum at a *slow rate* (*large spindle to small*), and take a

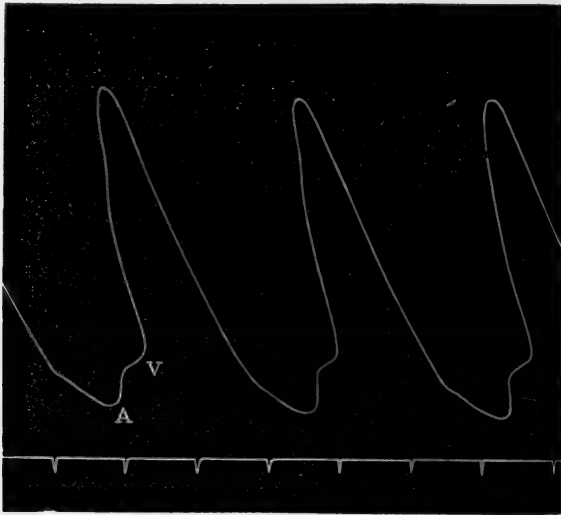


FIG. 58.—Contractions of the frog's heart.

A = auricular, V = ventricular contraction. The time is marked in seconds. The curve should be read from left to right. (The upstroke indicates contraction.) (L.H.)

record of several cardiac cycles, watching the heart to see which part of the trace corresponds to auricular and which to ventricular contraction. (If the downstrokes and upstrokes are too close together drive the drum on the fast gear from a small spindle on the shafting to a large one on the drum.) Remove the lever and put a time record in tenths of a second under the trace. Fix the tracing.

When fixed determine—

- (1) The rate of recurrence of the cardiac cycle, i.e. the rate of the heart;
- (2) the duration of the ventricular systole;
- (3) the duration of the auricular systole, if this is marked upon the trace.

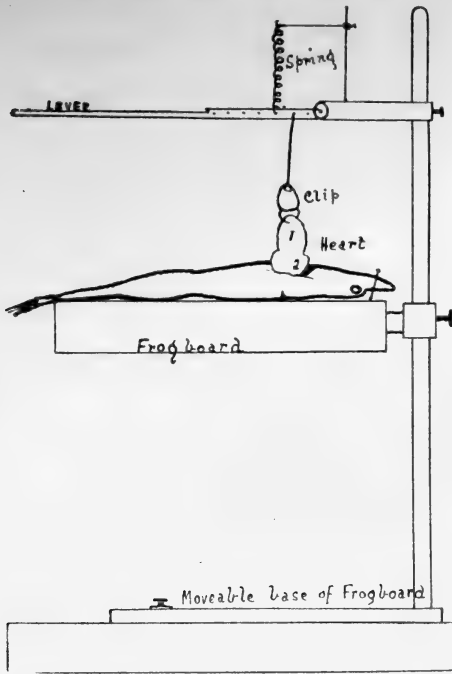


FIG. 59.—Arrangement of frog-board and lever for frog's heart.<sup>1</sup>

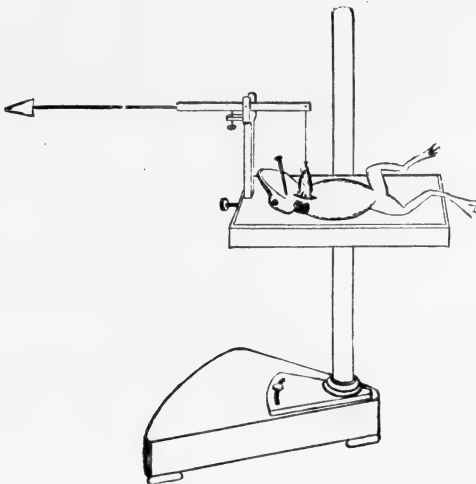


FIG. 60.—Lever for recording the frog's heart. (Pembrey and Phillips.)<sup>1</sup>

<sup>1</sup> With the form of heart lever (Fig. 59) the contraction is represented by the downstroke ; with the lever (Fig. 60) by the upstroke.

II. The Initiation of Contraction

What is the Influence of the Sinus? Stannius Experiment

By means of a needle, pass a piece of thread under the two aortæ, and, turning the ventricle forward, tie a loose loop between the auricles and the sinus (Figs. 61 to 64). When the heart has recorded a few contractions, swing the point of the lever off the drum, tie the loop *tightly* so as to separate the auricles from the

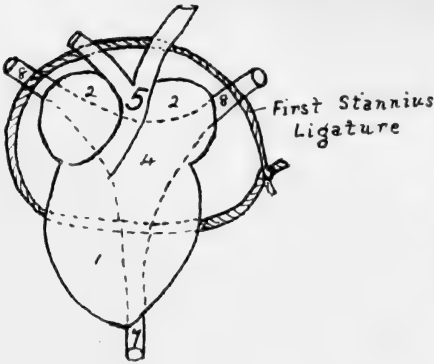


FIG. 61.

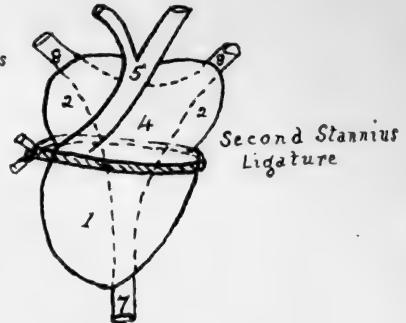


FIG. 62

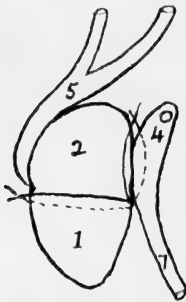


FIG. 63 (side view).

- 1. Ventricle.
- 2. Auricle.
- 3. Sinus.
- 5. Aorta.
- 7. Inferior Vena Cava.

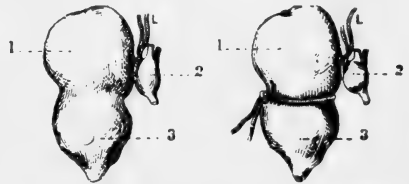


FIG. 64.—Stannius heart. The first and second ligatures (Hedon).  
1, Auricle; 2, Sinus; 3, Ventricle.

sinus, swing the point of the lever on and record the results. Has the sinus any important influence in initiating contraction?

Swing the lever off again and tie another loose loop round the auriculo-ventricular groove, and tighten it so that it exactly separates the auricles from the ventricle. Record the result and formulate your conclusion as to other parts of the heart besides the sinus having the property of initiating contraction (Fig. 65).



*Lesson XIV. To be provided for each pair of Students.*

1.—Frog heart apparatus. The end of the recording lever should have a glass writing point, preferably angled and swinging.—2. Recording Drum.—3. Time marker giving  $\frac{1}{10}$ th second.—4. Needle and thread.

## LESSON XV

**The Nervous Control of the Heart**

(1) Connect up the apparatus for giving a series of induced shocks (Neef's hammer), inserting a commutator (see Appendix), *without* crossed wires into the secondary circuit with two pairs of electrodes attached.

(2) Set a recording drum on the slow gear, and drive from small to large spindle.

*Always fit up the apparatus and test that it is working properly before commencing to prepare the animal.*

(3) Kill a frog and remove the brain *in front of* the tympanic rings by cutting the head across at that level. Then cut the spinal column and cord across between the shoulder blades and pith the lower part, thus leaving the medulla oblongata isolated and intact with the vagi passing from it to the heart.

**1. Intra-Cardiac Nervous Mechanism.**

Fix one pair of electrodes from the commutator by means of a pin to the cork so that their points touch the crescent, which may be seen as a white crescentic mark between the sinus and auricles on their *posterior* aspect (Fig. 52), and, with the point of the lever swung off the drum by means of the base-piece, stimulate by closing the key in the primary circuit and opening that in the secondary. If no change in the rate of the heart occurs, increase the strength of the current *till a marked change takes place*. Do not continue to stimulate after an effect is produced, but at once close the key in the secondary. When the rate of the heart has returned to the normal, swing the lever on and take a tracing as above, marking with a pin upon the drum when the stimulus was applied and when discontinued. Let the drum run till the previous rate of the heart is restored. Swing the lever off the drum. What conclusion do you draw from the result obtained ?

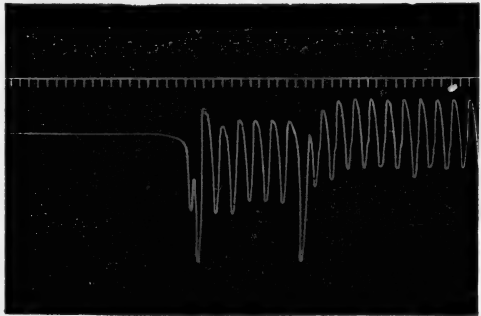


FIG. 65.—Contraction of the frog's heart.

The effect of tightening the first Stannius ligature at first gently and then firmly. The curve should be read from right to left. The time is marked in seconds. (L.H.)

## 2. Extra-Cardiac Nervous Mechanism.

Carefully insert the electrodes connected with the other side of the



FIG. 66.—Diagram of nerves in the frog's neck. Dissection from behind. (Pembrey and Phillips.)

G, Glosso-pharyngeal nerve; C, Carotid artery; Vs, Vago-sympathetic nerve; Hy, Hypoglossal nerve; Br, Brachial Plexus.

commutator into the medulla, fixing them on the cork of the frog-board by a pin. Tilt the bridge of the commutator to send the

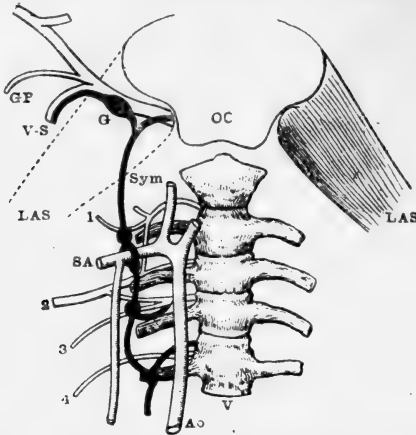


FIG. 67.—Diagram of the origin of the vago-sympathetic nerve (V.S.).

L.A.S.=levator anguli scapulae muscle. Ao.=aorta. 1, 2, 3, 4=first to fourth spinal nerves. Sym.=sympathetic nerve. G.P.=Glosso-pharyngeal nerve. G.=vagus ganglion. (Gaskell.)

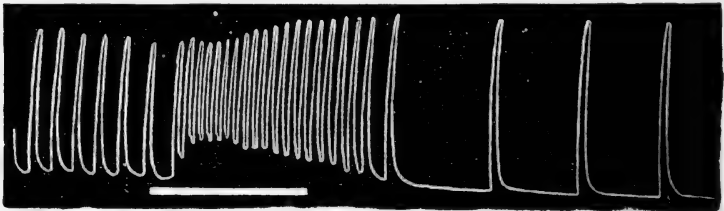


FIG. 68.—Contraction of the frog's heart. The effect of weak stimulation of the vago-sympathetic nerve.

The white line marks the duration of excitation. Note the latent time, the acceleration and increased tone and the after-effect. The curve should be read from left to right. (Pembrey and Phillips.)

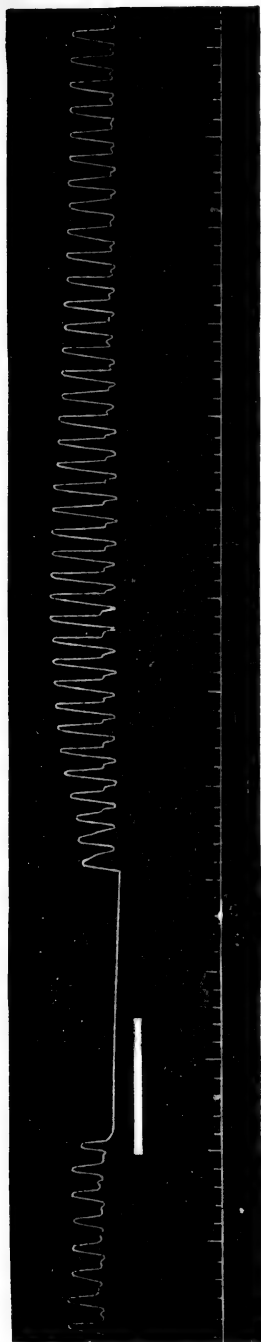


FIG. 69.—Contraction of the frog's heart. The effect of strong stimulation of the vago-sympathetic nerve. The white line marks the duration of excitation; the time is marked in seconds. The curve should be read from left to right. (Pembrey and Phillips.)

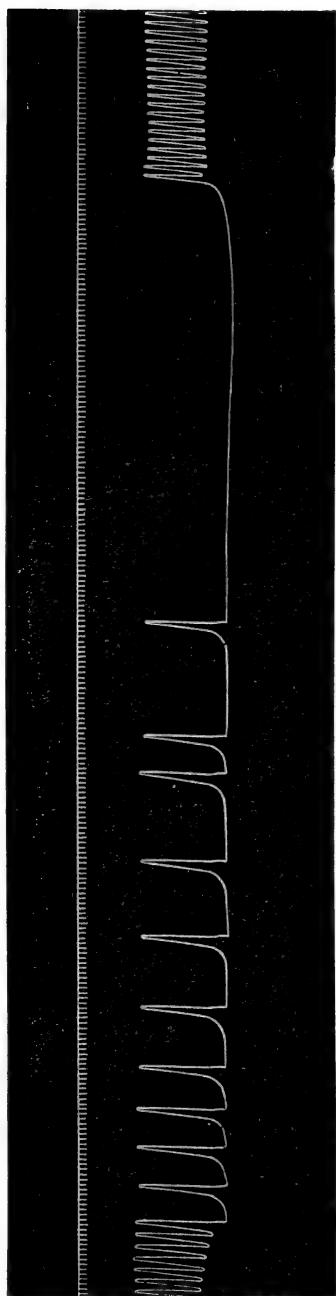


FIG. 70.—Contraction of the frog's heart. Excitation of the vago-sympathetic nerve between the points starred. Note the escape from complete inhibition. The time is marked in seconds. The curve should be read from right to left. (L.H.)

current through them, and, with the lever off the drum, stimulate and, if necessary, increase the strength of the current till a distinct effect is produced, stopping the stimulation whenever this occurs. When the heart again beats normally swing on the lever and start the drum. Again stimulate and take a trace, marking the moment of stimulation and of discontinuing it, and allowing the drum to run till the rate of the heart is restored. Formulate your conclusions.

### 3. Effect of Drugs.

Leaving the electrodes in position as in 1 and 2, paint the heart with 0.1 per cent. solution of atropine sulphate. Allow two minutes to elapse and then stimulate (1st) the crescent, (2nd) the medulla.

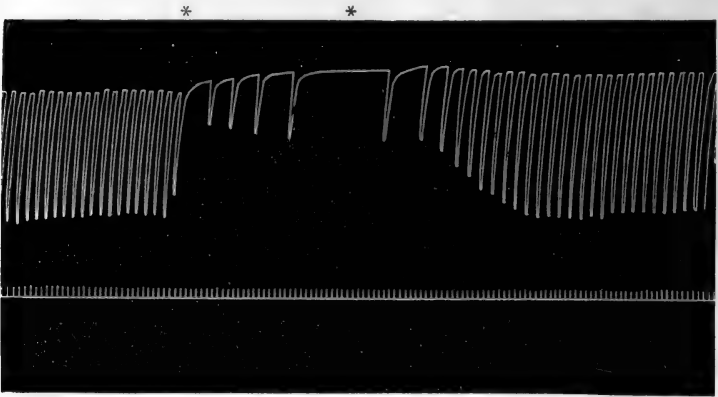


FIG. 71.—Excitation of vago-sympathetic.

Note the after effect—a staircase augmentation of the heart-beat. The stars indicate the beginning and end of stimulation. The downstroke represents contraction. The time is marked in seconds. (L.H.)

Note any difference from the previous reaction. Take a tracing and formulate your conclusion. Run off a time trace in seconds and fix the tracings. The experiment may be tried using nicotine 1 in 1000 saline solution.

**Is the Heart's Action Automatic?** Excise the heart *with the sinus attached*, and place it in a watch glass and study its movements, counting the number of beats per minute.

**Influence of Temperature.**—Now place the watch glass upon ice and observe the effect. When a marked change in the rate has taken place and been recorded, remove the watch glass from the ice and place it upon the palm of the hand and record any change in the rate.

The influence of temperature may be investigated before trying the effects of drugs by pouring over the heart normal saline solution

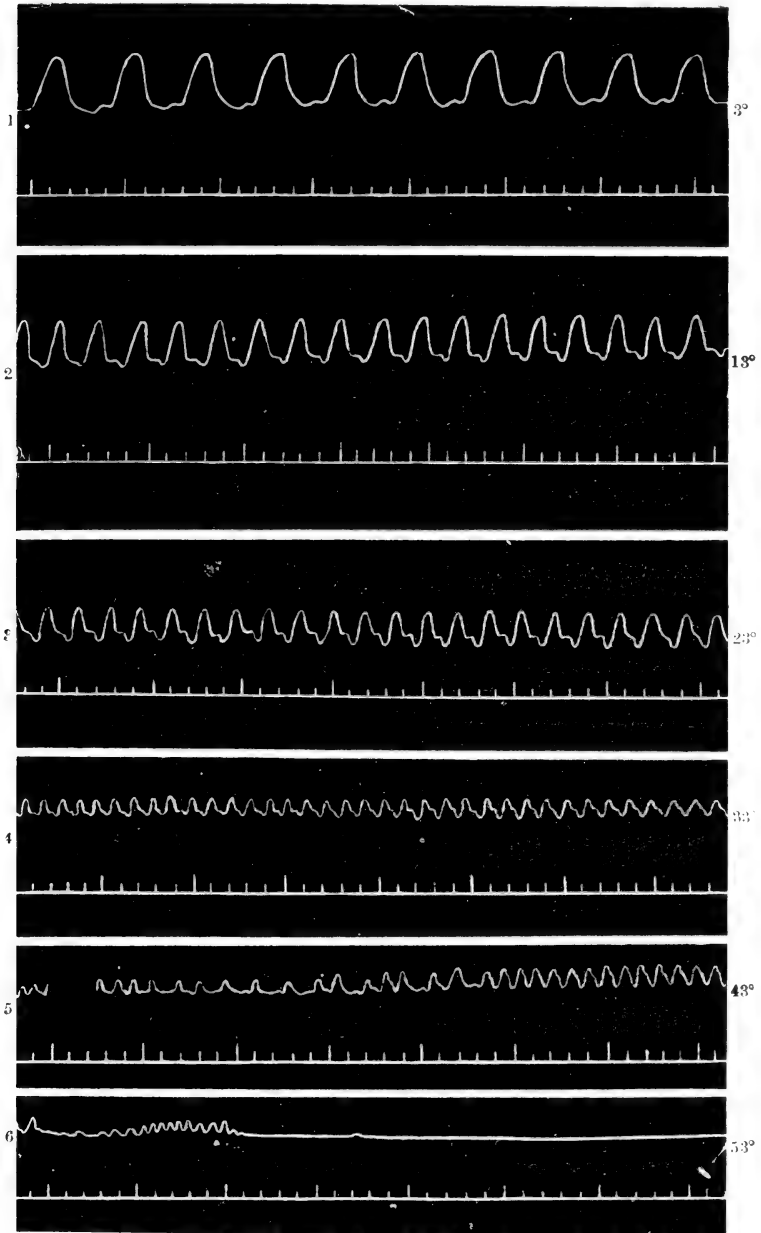


FIG. 72.—Contraction of the frog's heart recorded by the suspension method. Effect of pouring over the heart normal saline at the temperatures indicated. The water cools rapidly when this method is used, and the heart is not heated throughout its mass to the temperature indicated. (Pembrey and Phillips.)

at about 3°, 13°, 33° C., and recording the contractions on a slow drum. (Fig. 72.)

(Read the Nervous Control of the Heart in Text Book.)

*Lesson XV*

Apparatus, etc., given for Lesson XV. (p. 65).

5. Induction coil with wires, electrodes, keys, etc.—6. Atropine sulphate solution, 0·1 per cent.—7. Nicotine solution, 0·1 per cent.—8. Watch-glass.—9. Broken ice.

## SECTION IV

# RESPIRATION

### LESSON XVI

#### 1. Changes in the Chest during Breathing

(a) With a tape, measure the circumference of the chest of a companion in full expiration and in full inspiration and record the result.

(b) With the *cyrtometer* provided take a tracing of a section of the chest in expiration and in inspiration and compare them, measuring and recording the diameters—(i) close up under the armpits ; (ii) at the base of the lungs, about the level of the eighth or ninth rib. (*Keep the records.*)

(c) Now place the middle finger of the left hand flat on the sixth right intercostal space in front of the chest and strike it firmly with the middle finger of the right hand. Do this during expiration and during inspiration, and note any difference in the sound produced. The air-containing lung yields a resonant note, the solid liver yields a dull note. Record your conclusion as to the vertical extent of the lung in expiration and in inspiration.

(Read Text Book on the Movements of the Chest.)

#### 2. Changes in the Air breathed

(1) Breathe upon a piece of cool clean glass. Note what happens and draw a conclusion as to the saturation of expired air.

(2) Breathe out repeatedly through the vessel of lime water provided, and note the change produced. What is this due to ?

(3) Breathe out repeatedly through the weak solution of potassium permanganate provided, and note the change produced. What is this due to ?

(4) A bell-jar, fitted with a cork through which passes a glass tube to which is attached a piece of rubber tubing with a glass mouth-piece is provided. It rests on a glass plate lubricated with vaseline. Place a lighted candle under the bell-jar and breathe the air of the jar through the tube. Note the changes in the flame and explain them.

#### 3. Sounds produced during Breathing

With a stethoscope listen (a) over the windpipe and (b) over the

middle of the right side of the chest in the axillary line while the person breathes, and describe the sounds heard at each place, timing their relationship to inspiration and expiration.

(Read Text Book on the Mechanism of the Breath Sounds.)

#### 4. The Rate of Respiration

Count the number of respirations in a person who has been and is sitting still and whose attention is directed to something other than his breathing, and again in the same person after taking violent exercise.

#### 5. Collapse of the Lungs when the Thorax is Opened

Distend the rabbit's lungs provided by blowing into the trachea and then observe their elastic collapse. Measure the force of this with a water manometer.

Examine a section of lung stained with orceine to show the elastic tissue, and note its abundance.

#### 6. Recording the Movements of Respiration

(a) To record the *movements of the air*. Arrange a slowly moving drum by putting the drum on the slow gear and connecting a small spindle on the shafting to the largest on the drum. Connect a recording tambour by means of a piece of rubber tubing with a short piece of glass tube. Push the movable base-piece of the stand against the stop, and by moving the whole stand bring the point of the lever lightly against the drum. Insert the glass tube into one nostril, breathe with the mouth closed, and record the movements of the lever. The upstroke and downstroke should occupy about 1 inch on the drum. Adjust the speed of the drum to secure this. When a trace has been taken for about a minute, swing the lever off the drum by means of the movable base-piece, put a time record in seconds on the drum and measure the duration of inspiration and of expiration.

The record should be taken with the subject sitting still, and not looking at the drum.

(The precautions as regards disinfecting the nasal tube, p. 12, note, must be observed.)

(b) To record the *movements of the chest wall*. Insert in the course of the rubber tube a glass T tube with a clamp. Connect the glass tube with a toy balloon, and place it under the waistcoat or a bandage round the chest, and slightly distend the balloon. Take a tracing.

#### The Influence of Carbon Dioxide on Respiration

Put the drum on a *very* slow speed so that in ordinary breathing the upstroke and downstroke are just separate from one another on the tracing.

(1) While taking a trace of normal breathing by means of a tube in the nostril, hold the breath till a marked desire to breathe again



is experienced ; then allow respirations to recur and be recorded. Measure the length of the absence of breathing. Stop the drum.

Now breathe deeply and forcibly for about 2 minutes, so as to clear the  $\text{CO}_2$  out of the blood, starting the drum and thus recording the last two or three respirations. Then hold the breath as above, and measure the length of the absence of breathing, and compare it with the last. Another student should observe any change in the appearance of the face, and must count the pulse for half a minute before and again after the forced breathing.

(2) Count the pulse and record its rate. Run several times up and down stairs to increase the  $\text{CO}_2$  in the blood, and again record the respirations from the nostril and count the pulse, recording the rate. Compare the tracing and the pulse rate with those taken at rest, and draw your conclusions as to the influence of muscular exercise on the breathing and on the rate of the heart.

(Read Text Book on the Regulation of Breathing.)

(IV. of Lesson XIII. is best done at this time.)

*Lesson XVI. To be provided for each pair of Students.*

1. Measuring tape.—2. Cyrtometer.—3. Piece of glass.—4. Vessel of lime water.—5. Vessel with very dilute potassium permanganate.—6. Bell-jar with candle and tube attached for breathing through.—7. Stethoscope.—8. Lungs of rabbit and cat with tube tied in trachea.—9. Marey's recording tambour on stand with tube and small balloon fitted to glass tube : a T tube with clamp is useful.—10. Recording drum.—11. A vessel of lysol solution.—12. Water manometer.

*For Class.*

Microscope with section of lung stained with orcein to show the elastic tissue.

## SECTION V

### MISCELLANEOUS

#### LESSON XVII

##### I. DIGESTION

###### **Swallowing.**

1. Swallow a mouthful of water or saliva and, with the finger, determine the changes in the position of the tongue, hyoid bone and larynx.

2. Swallow some milk or some water coloured with methylene blue, and then examine the posterior aspect of the epiglottis with the laryngoscope (p. 11).

3. Try to swallow with the mouth empty, and note the result.

4. Listen with a stethoscope over the left side of the 10th dorsal vertebra when a large mouthful of water is being swallowed, and note the sounds that are heard. *These, like the breath sounds (p. 73), are best heard with the clothes removed.*

5. Try this again after a rapid series of swallows.  
(Read Swallowing in Text Book.)

###### **Passage of intestinal contents through the ileo-cæcal valve.**

About 4 to 6 hours after breakfast listen for some time with a binaural stethoscope in the right iliac region, and note any sounds observed.

##### II. TEMPERATURE

*The student should provide himself with a one-minute clinical thermometer.*

1. (a) Take the temperature in the axilla and in the mouth, and (b) take the temperature in the mouth night and morning for a week, and record your results on a chart.

2. Take the temperature of (a) the palm of the hand, and (b) the anterior surface of the chest, placing the thermometer under a layer of flannel, and (c) take the temperature between the shirt and waistcoat.

3. Take the temperature in the mouth and axilla after a spell of

violent muscular exercise, and repeat the observations at 5-minute intervals till it is again normal.

(Read Temperature Regulation in Text Book.)

### III. THE ESSENTIAL NATURE OF LIVING MATTER IN ITS SIMPLEST FORM

1. **OBJECT.**—To learn something of the essential nature of living matter (protoplasm).

2. **METHOD.**—Take a very simple form of living matter—the yeast plant—and place it under various conditions. Place a small quantity of yeast on a slide, and add a drop or two of water. Rub up into an emulsion with a glass rod, and transfer a little on the end of the rod to: (A) a test-tube of a solution containing the chemical elements which occur in the yeast, C.H.O.N.S. and P., e.g. urea  $\text{CO}(\text{NH}_2)_2$ , glucose  $\text{C}_6\text{H}_{12}\text{O}_6$ , with traces of sodium phosphate  $\text{Na}_2\text{HPO}_4$ , potassium sulphate  $\text{K}_2\text{SO}_4$ , and calcium phosphate  $\text{Ca}_3(\text{PO}_4)_2$ ; (B) a test-tube filled with water.

See that the tubes are quite full. Shake well and examine a drop with the microscope, and make a rough estimate of the number of torulæ in two or three fields of the microscope. Draw one or two torulæ.

#### At each Bench—

Students at places 1 and 2 at once insert the corks firmly into the tubes. The tubes of 1 are placed in an incubator at  $37^\circ\text{C}$ . The tubes of 2 are placed in a vessel of broken ice.

Students at place 3 introduce a few drops of phenol solution, insert the cork, and place the tubes in the incubator.

Students at place 4 boil the tubes before quite filling them, cool them under the tap, fill them with water, insert the corks, and place them in the incubator.

3. **RESULTS.** (A) **ON YEAST.**—Next day the tubes are to be examined with the naked eye before and after shaking, and the condition of each tube studied, contrasting it with the condition on the previous day. A drop of the fluid after shaking is to be examined with the microscope, and the number of torulæ in two or three fields to be estimated.

The students at each bench should make a combined table of their results as to gas-formation, change in opacity, and change in number of torulæ, using + and - as signs.

	Gas.	Opacity.	Number.
Tubes with sugar (1) at .	$37^\circ\text{C}$ .		
"    "    "    (2) at .	$0^\circ\text{C}$ .		
"    "    "    (3) with	Phenol		
"    "    "    (4) . .	Boiled		
Tube with water (5) at .	$37^\circ\text{C}$ .		

(B) ON FLUID.—(a) *Disappearance of Sugar. (Demonstration.)* The original solution (A) and the solution after incubation (B) have been boiled for some time with phenylhydrazine and acetic acid, which forms a yellow insoluble compound with glucose. Note the difference in the amounts in (A) and (B).

(b) *Formation of Alcohol.* Some of the fluid from tubes 1, after being in the incubator, is distilled. To about half an inch of the distillate, add a few drops of potassium bichromate and a little dilute sulphuric acid and warm. Note:

1. Pungent odour—Aldehyde.
2. Green colour.

(c) *Nature of Gas evolved. (Demonstration.)* With a fine pipette add KHO dissolved in alcohol to a Doremus' ureometer in which the solution has been incubated with yeast, and note the absorption of the gas evolved—CO<sub>2</sub>.

4. CONCLUSIONS.—(1) What has happened to the yeast in each of the tubes?

(2) What conclusions do you come to as to the influence upon the yeast protoplasm of the various conditions to which it has been subjected?

(3) How has the growth of the yeast taken place?

(a) Where does yeast protoplasm get material for growth?

(b) Where does yeast protoplasm get energy for growth?<sup>1</sup>

<sup>1</sup> For Enzyme Action, see Chemical Section.

## APPENDIX

### THE USE OF ELECTRICITY FOR STIMULATING IN PHYSIOLOGY AND MEDICINE <sup>1</sup>

(The Student should revise his knowledge of Electricity.)

**Sources of Electricity most Generally Used.**—In hospitals the town supply of electricity is generally used either directly, the voltage being reduced by means of a resistance, or better by a motor generator, or indirectly by charging accumulators.

The wires used to lead off the current are connected with screws upon a SWITCH-BOARD. There are generally two sets of terminals, one for using the galvanic current, one for using the faradic.

In some physiological laboratories a similar installation is used.<sup>1</sup>

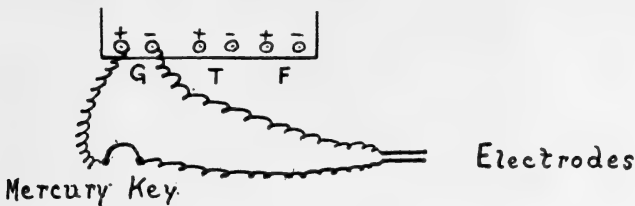


FIG. 73.—Simple Galvanic Current.

Fig. 73 shows a simple form of switch-board with terminals G + and — (anode and cathode) for using the galvanic current; terminals F for using the faradic current, and terminals T for recording intervals of time. These last are connected with a wheel chronograph, driven by a motor, which can be set to give intervals of  $\frac{1}{10}$ th or 1 second.

<sup>1</sup> In the University of Glasgow the current is supplied from an accumulator giving from 15 to 30 volts to the switch-boards on the tables. The terminals on the switch-board marked G are for galvanic stimulation, and give from 0 to 400 milliamperes. The lever regulator on the switch-board controls the strength of the current (from the G terminals only). When placed at W it gives the weakest current, when moved towards S it gives the strongest current. The terminals marked F are for Faradic stimulation, and give about 1.5 amperes. The terminals marked T are connected with a chronograph and interrupt at 1 sec., or 0.1 sec., according to the way in which the instrument is set.

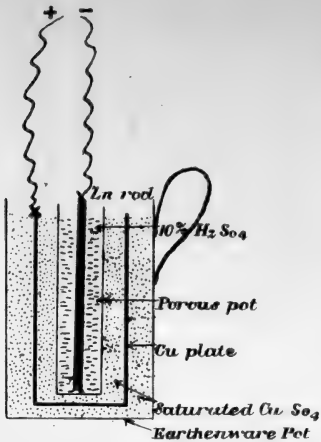


FIG. 74.—Diagram of a Daniell cell seen in section.

In this figure the lever for varying the strength of the galvanic current is not shown.

If such an installation is not available GALVANIC CELLS must be used either singly or in series. So many different kinds are now in use that it is unnecessary to describe them here. Probably the Daniell cell (Fig. 74) is the most suitable for physiological purposes on account of the steadiness of the supply of current and of its uniform voltage.

The strength of the current may be varied by inserting into the circuit some form of RHEOCORD, such as is shown in Figs. 75, 76, 77.

The ends of the wires are generally provided with some form of ELECTRODES for application to the tissues. These may consist of a couple of pins thrust through

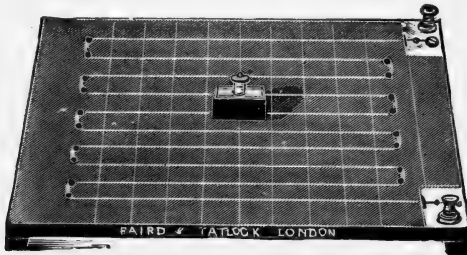


FIG. 75.—Simple form of monochord.

a piece of cork or vulcanite (Fig. 78), or they may be guarded so as to prevent contact with surrounding tissues by being inserted

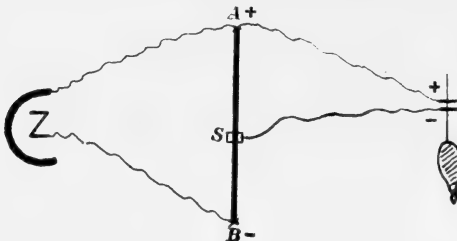


FIG. 76.—To illustrate the principle of the monochord.

through a piece of vulcanite with a notch over the ends of the wires (Fig. 78).

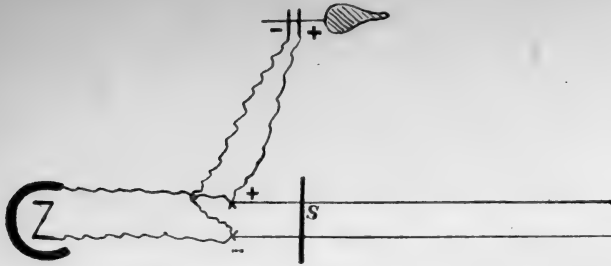


FIG. 77.—The rheocord arranged to vary the strength of a current passing through a nerve.

It consists of two parallel wires connected by a movable metal slider S. By moving the slider S to the right the resistance of the rheocord in circuit and therefore the amount of battery current passing through the nerve would be increased.

If an electrode is to be applied to the skin, it should have a large surface covered with chamois leather saturated in a strong solution



FIG. 78.—Two forms of electrodes.

of common salt in order that the resistance of the skin may be overcome.

In certain physiological investigation non-polarisable electrodes



FIG. 79.—A mercury key.

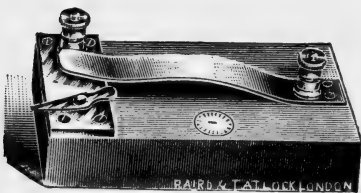


FIG. 80.—A spring key.

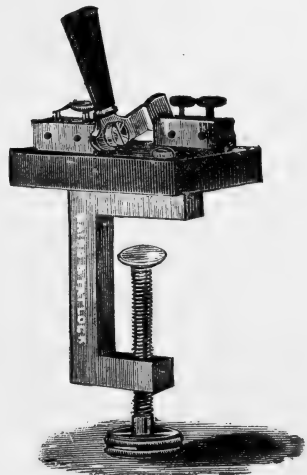


FIG. 81.—A friction or Du Bois key.

have to be used (see Part II.), but these do not concern the junior student.

The current is thrown into or cut out of the structure to be



FIG. 82.—Plan of the use of a Du Bois key, as a simple make and break key.

acted upon (*made* or *broken*) by means of some form of KEY such as those shown in Figs. 79, 80, 81 (arranged in the circuit as shown in Figs. 82, 83, 84).

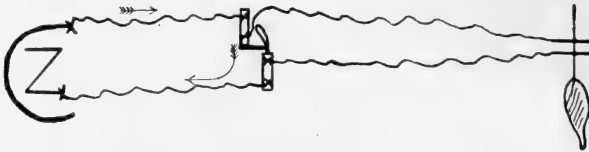


FIG. 83.—Arranged as a short-circuiting key : key shut.

They may be used either to make the current when closed in the circuit (Fig. 82), or to short-circuit the current back to the battery when closed (Fig. 83).

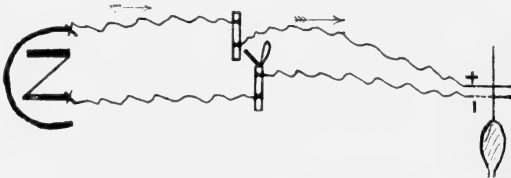


FIG. 84.—Arranged as a short-circuiting key : key open.

### I. The Use of the Galvanic Current.

**To Test the Action of the Current.**—Connect thick covered wires with the terminals marked *G* on the table, + for positive (anode) and - for negative (cathode) from the constant current supplied from a generator, or connect them to the terminals of three Daniell cells in series. Insert into the circuit a *mercury key*, as shown in the diagram, so that when it is closed the current will flow through the terminals (Fig. 73). Hold the ends of the wires, one on each side of the tongue, and note the sensations produced when the current is allowed to pass (*made*) by closing the key, when it is cut out (*broken*) by opening the key, and when the current is flowing. Note whether the sensations are different or similar at the two poles on closing and on opening and during the flow.



Taking sensation as the index of stimulation, record on the table below the results of the sudden making and breaking of the electric current, and of its continuous flow.

	Make.	Flow.	Break.
Anode . . .			
Cathode . . .			

II. Faradic Current. The Use of the Induction Coil.

1. **Single Induced Shocks.**—An ordinary type of induction coil or inductorium is shown in Fig. 85.

Lead off wires from the screw-terminals *F* on the table (Figs.



FIG. 85.—An induction-coil.

86 and 87) or from a galvanic cell (Fig. 87) to the screws on the top of the end of the primary coil of an induction coil (Fig. 86), and introduce a *mercury key* into the circuit. Lead wires from the

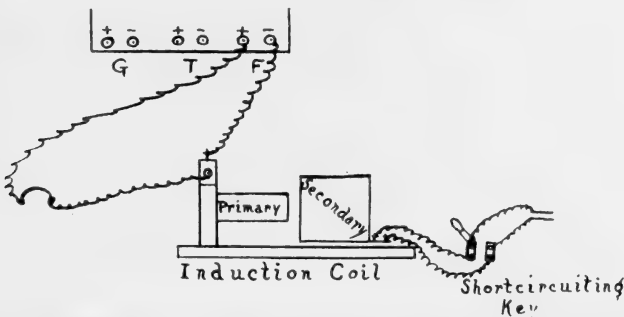


FIG. 86.—Arrangement for single induction shocks, using electrical supply from switch-board.

terminals of the secondary coil to a *friction key*, so that when it is closed the current is short-circuited (Figs. 86 and 87). Lead off the wires of a pair of pin electrodes from this key. Pull the secondary coil well away from the primary, open the friction key, so that the

current may pass to the electrodes, and use them as the wires in the last experiment. Note the effect on the tongue of the sudden appearance and disappearance of the current induced in the

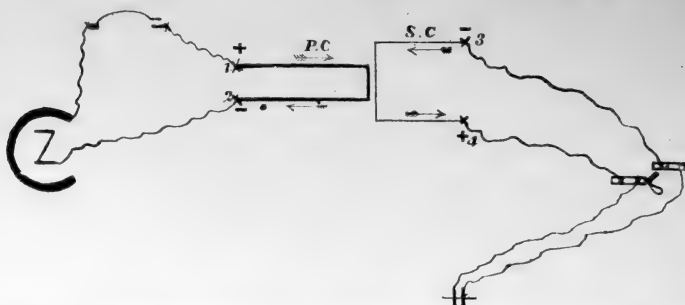


FIG. 87.—Arrangement for single induction shocks, using galvanic cell.

secondary coil each time the primary circuit is made or broken by means of the mercury key.

The experimenter can thus apply induced shocks at will.

The results of making and breaking the primary circuit may be recorded on the following form :—

Making Primary.	Breaking Primary.

**2. A Series of Induced Shocks.**—Add to the primary circuit fitted up as above a vibrating spring and a mercury cup (Fig. 88). At

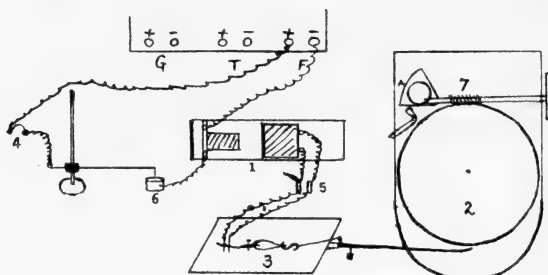


FIG. 88.—Series of Induced Shocks.

- |                    |   |
|--------------------|---|
| 1. Induction Coll. | 5. Friction Key.                                    |
| 4. Mercury Key.    | 6. Vibrating Spring with Cup<br>containing Mercury. |

each vibration the spring makes and breaks the circuit. The rate of vibration can be varied by altering the length of the spring.

The experimenter can thus apply a regular series of induced shocks at any given rate.

**3. A Rapid Series of Induced Shocks (Neef's Hammer).**—Connect up an induction coil with the screw terminals marked *F*, inserting

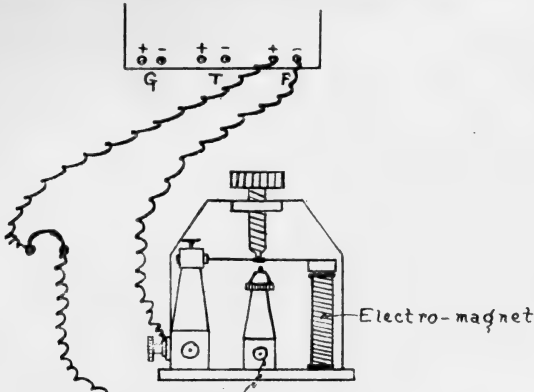


FIG. 89.—End of Coil showing Neef's hammer.

the wires into the binding-screws *in the pillars* at the end of the primary coil, so as to bring the Neef's hammer into action (Fig. 89). (Note.—Some of the coils are of a different pattern and have only

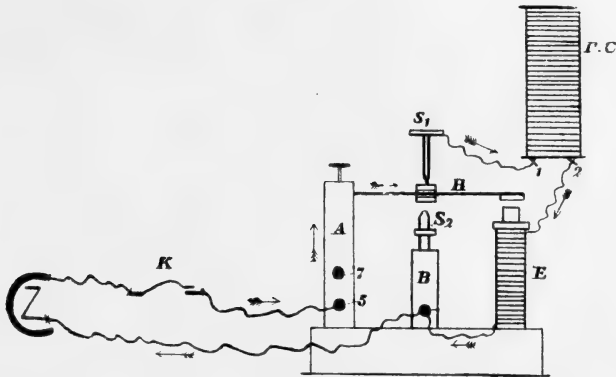


FIG. 90.—Diagram to show the action of Neef's hammer.

one pillar. In this case connect the second wire to the upper lateral terminal.) Introduce a mercury key into the primary circuit and a friction key into the secondary circuit as before (Fig. 86).

Note that, when the mercury key is closed, the current passes round the electro-magnet, which pulls down the hammer and spring, and thus breaks the contact at the screw in the middle of the spring. The current is interrupted, the magnet demagnetised

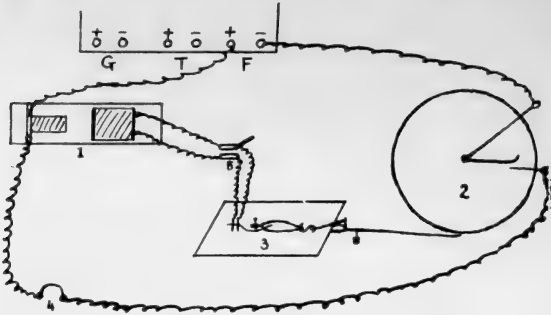


FIG. 91.—Single Induced Shocks at a given Instant.  
1. Induction Coil. 2. Drum. 4. Mercury Key. 5. Friction Key.

and the spring bounds back. The number of interruptions depends on the length of the spring.

Open the friction key and pull the secondary coil well away from the primary and apply the wires to the tongue. Close

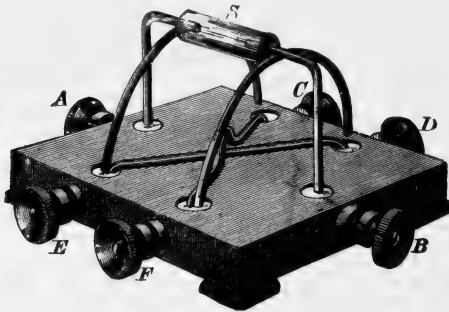


FIG. 92.—A Commutator on Pohl's Reverser.

*A* and *B* the two side cups; *C*, *D*, *E* and *F* the four corner cups;  
*S*, the handle made of glass or vulcanite.

the mercury key, and if no sensation is experienced push the secondary coil nearer to the primary. Record the resulting sensation.

**4. A Single Induced Shock at a Given Instant.**—Insert the recording drum into the primary circuit fitted up as in Fig. 86 (Fig. 91). To

do this connect wires to the terminals at its base. One of these is insulated and the drum consequently acts as an interrupter of the primary circuit except at the instant at which the striker makes contact with the knife edge projecting from the insulated terminal.

By this means one induced shock (make and break) is given at a fixed point in each revolution of the drum.

### III. The Use of the Commutator.

The commutator, one form of which is shown in Fig. 92, consists of six mercury pools arranged in three pairs and connected with terminals. The centre pair can be connected at will by means of a movable bridge with *either* the left-hand pair *or* the right-hand pair (Fig. 93).

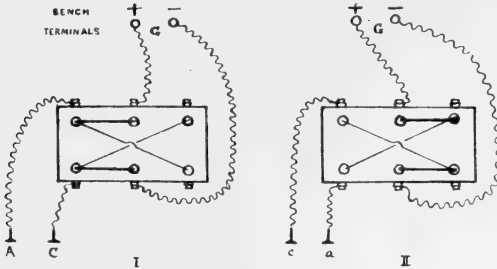


FIG. 93.

A. It is used to reverse the direction of the current in the circuit. Connecting the diagonally opposite pools at either end are cross wires insulated from each other.

The current is led from the electric supply to the middle pools.

(i) When the bridge is placed as in Fig. 93, I, the current from the positive terminal follows the metallic circuit and reaches electrode A direct. If now the gap between A and C is bridged the current flows into the left lower pool, across the bridge to the lower middle pool and back to the negative terminal on the bench.

(ii) When the bridge is placed as in Fig. 93, II, the current from the positive terminal passes to the right upper pool, then along the diagonal wire to the left lower pool and reaches electrode a. On bridging the gap the current flows to the left upper pool along the wire to the right lower pool, across the bridge to the lower middle pool and so back to the negative terminal on the bench.

Thus the electrodes are transposed, anode becoming cathode and cathode, anode.

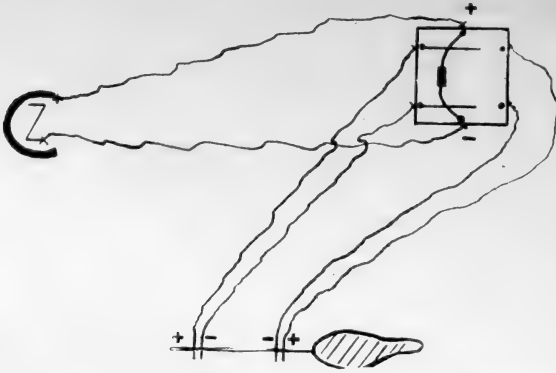


FIG. 94.—Plan of the arrangement of the two alternative circuits.

*B.* It may be used to pass the current through either of two pairs of circuits. By removing the cross wires and connecting a pair of electrodes to each end pair of terminals a current may be passed through *either* the left-hand pair *or* the right-hand pair by moving the bridge. In this case the instrument acts simply as a two-way key (Fig. 94).

EXPERIMENTAL PHYSIOLOGY

ADVANCED COURSE

BY

M. S. PEMBREY





# ADVANCED EXPERIMENTAL PHYSIOLOGY

## CHAPTER I

### THE PROPERTIES OF NERVE

Although the nerve is not a unit, but a branch of a nerve-cell which conducts an impulse to, or from, the periphery, it is convenient to examine its characteristics apart from its nerve-cell. The chief of these are **excitability** and **conductivity**. Excitability, or, as it is sometimes called, *irritability*, is the response to a stimulus; a nervous impulse, the real nature of which is unknown, is started at the point stimulated, and is transmitted or conducted along the nerve.

Nerves can be stimulated by electrical, mechanical, chemical or thermal agents; of these the most important in experimental physiology is the electrical, for it can be finely graduated, is of extremely short duration, and can be applied repeatedly without damage to the nerve, but its use is not free from sources of error.

**Electrical Stimulation of Nerve.**—An induction-apparatus is arranged for single induction-shocks and a simple pair of electrodes is connected with the secondary coil by means of a Du Bois key. A preparation of the sciatic nerve in its entire length and of the gastrocnemius muscle of a pithed frog is made, and near the origin of the nerve is applied the pair of electrodes. On the passage of an induction-current through the electrodes the nerve is stimulated, and an impulse is sent down the nerve, reaches the muscle, and causes it to contract. This is *indirect* stimulation of the muscle, and is, if a weak current be used, not due to an escape of the electric current along the nerve towards the muscle. This should be proved by the following experiment. A moistened thread is tightly tied round the nerve at a point between the electrodes and the muscle. The passage of a weak induction-current of the same strength as that previously used will stimulate the upper portion of the nerve, but the nervous impulse will not pass through the block produced by the thread. A breach in the physiological continuity has been produced, and the nervous impulse is not conducted through the ligatured nerve. The moistened thread

would not prevent the passage of a purely electric current. A loss of excitability readily occurs if the nerve is allowed to dry, but during this process there may be irregular fluctuations in the excitability above and below the normal.

**Unipolar Excitation.**—Connect a battery to a coil so as to give tetanising shocks; connect a wire to one pole of the secondary coil and place its free end on the tongue. If the secondary coil be moved completely over the primary, faint shocks will be felt. The explanation of this phenomenon is that the making and breaking of the primary circuit causes free electricity to collect at the end of the wire connected with the secondary coil; when the electromotive force of this charge is sufficient to overcome the resistance of the tissues of the body, the circuit is completed through the body, the floor and desk, and so back to the other pole of the secondary coil. With the wire still on the tongue, touch the other pole of the secondary coil with a moistened finger; much more powerful shocks are felt because a more direct circuit from one pole to the other of the secondary coil has been provided.

Repeat the experiment on a sciatic-gastrocnemius preparation in the following way, with either tetanising or single-induction shocks. Lay the preparation on a perfectly clean and dry glass-plate and place a wire connected with one pole of the secondary coil under the nerve; no contraction of the muscle takes place because the dry plate insulates the preparation and the secondary circuit cannot be completed. Now touch the muscle with a wire, the other end of which rests on a gas or water pipe; the muscle contracts because the circuit is completed through the earth. It is not even necessary that the conductor should touch the preparation, for, if a moistened finger is brought as near the muscle as possible without touching it, the muscle contracts, especially if a moistened finger of the other hand touches the other pole of the secondary coil. In this case the human body acts like a condenser charged with electricity, which by its approach can stimulate muscle or nerve. Further, if the nerve be ligatured between the electrode and the muscle, or cut across and the two cut ends laid over each other, which will prevent the passage of a nervous impulse along it, contraction of the muscle is still produced, because the discharge takes place along the whole length of nerve and muscle between the electrode and the point by which the muscle is connected to the earth, so that any irritable tissue in the course taken by the charge is stimulated.

If, however, the muscle and nerve preparation is laid on an ordinary moistened muscle-board, the insulation is so slight that one electrode, connecting the nerve and the secondary coil, will by itself cause the muscle to contract.

It is in order to guard against accidental stimulation of muscle and nerve by unipolar action that a Du Bois key must always be placed in the secondary circuit, and must always be kept closed except when the tissue is being intentionally stimulated. The

brass bridge of the key, which has many thousands of times less resistance than the tissue<sup>1</sup> between the electrodes, affords a perfect closure of the secondary circuit and prevents static electrification of the electrodes.

Errors from unipolar action are liable to take place, especially in the study of the electromotive phenomena of muscle and nerve by the electrometer and galvanometer.

**Galvani's Experiment with Metals.** A piece of zinc is connected with a piece of copper wire and the free end of one element is placed under the sciatic nerve of a muscle-nerve preparation. If the circuit be completed the nerve will be stimulated and the muscle contract at *make*; on breaking the circuit there will be a contraction at *break*.

**Mechanical Stimulation** of the nerve can be shown by pinching the nerve with a pair of forceps; the muscle contracts, showing that a nervous impulse was produced. Such a method of stimulation injures the nerve, but by means of simple arrangements a nerve can be stimulated mechanically without damage. A light hammer worked by an electro-magnet may be used to tap the nerve (see p. 128), or small drops of mercury from a funnel may be allowed to fall upon the nerve. Such methods are useful in experiments (see p. 128) in which an electrical stimulus might introduce a source of fallacy, but for ordinary experiments they are undesirable, since there is a difficulty in maintaining a constant strength of stimulus, and there is a danger of damage to the nerve.

**Thermal Stimulation** is next shown by the application of a hot wire to the nerve. The muscle contracts. The damaged portion of the nerve is cut away, and to the end of the living nerve is applied a crystal of common salt; the muscle soon shows irregular twitches due to the **chemical stimulation** of its nerve (see p. 127). The last form of stimulus is obviously limited to special experiments, for the stimulus is not easily graduated and damages the nerve.

## CHAPTER II

### THE CONDITIONS WHICH AFFECT SINGLE MUSCULAR CONTRACTIONS

(a) **Different Muscles.** (b) **Veratrine.**—The curve produced by the contraction of a muscle may be altered not only by such influences as temperature, load, fatigue, and drugs, but also by the differences in structure of various muscles. The muscular fibres of the frog are found to present two varieties, clear and granular, which differ both in structure and in physiological properties. The gastrocnemius may be taken as an example of a muscle whose

<sup>1</sup> The resistance of a piece of a frog's sciatic nerve 1 cm. long is about 100,000 ohms.

fibres consist largely of the clear variety, and the hyoglossus of the granular variety, i.e. a muscle in which the majority of muscle-fibres contain more nuclei and are relatively richer in undifferentiated living material, the sarcoplasm. The chief physiological differences between granular and clear muscles are, that granular muscles have a slower and more prolonged contraction, are less excitable, more easily tetanised, and less readily fatigued.

In mammals the same differences between red and white muscles can be shown to exist. Red muscles, such as the masseter or soleus of the rabbit, differ structurally in having more sarcoplasm and nuclei in their fibres, and are redder in colour owing to a much richer capillary network between their fibres and to the presence of myohaematin in the fibres themselves; physiologically they are far less readily fatigued and show a contraction four or more times as long as that of the white gastrocnemius (Fig. 95). The experiment can be made after the rabbit has been killed by breaking its neck.

For comparison with the single twitch of the gastrocnemius, that given by the hyoglossus may now be studied. This muscle, arising from the anterior edge of the body of the hyoid cartilage, runs forwards into the substance of the tongue.

A **Hyoglossus Preparation** is made by cutting off the whole of the lower jaw, including the tongue and hyoid cartilage. Place it on the myograph board, mucous surface upwards, turn the tongue forwards, and connect its tip to the lever by a thread. Firmly fix the hyoid cartilage by running a pin through it into the cork. Two needle electrodes transfix the base of the muscle just in front of the hyoid.

All the other connections are the same as when studying the single contraction of the gastrocnemius; a weight of 5 or 10 grams is placed near the axis of the lever.

Compared with the single twitch of the gastrocnemius, that given by the hyoglossus (Fig. 96) shows the following differences: the whole contraction lasts more than twice as long, the latent period is slightly longer, but it is the period of shortening and still more that of relaxation which is more gradual and prolonged.

**Action of Veratrine.**—A brainless frog is poisoned by injecting into the dorsal lymph sac 5 minims of a saturated (1 in 1000) solution of veratrine in normal tap-water saline. In order that the drug may be rapidly absorbed it is important not to “pith” the frog, but to destroy its cerebrum with a pair of Spencer-Wells pressure forceps. In about ten minutes it will be observed that the hind legs are very slowly and imperfectly flexed after a jump, and a few minutes later the frog will be seized by a spasm when it jumps. As soon as these symptoms appear the remaining portions of the central nervous system are destroyed, and a sciatic and gastrocnemius preparation made.

In the meantime the action of veratrine may be studied on the

hyoglossus preparation used in the previous experiment. Five minims of the veratrine solution are injected into the lymph sac in which the muscle lies. The drum is arranged to revolve at a slow rate of about 2 cm. in 10 secs., and a simple key instead of the "striker" of the drum is placed in the primary circuit. After

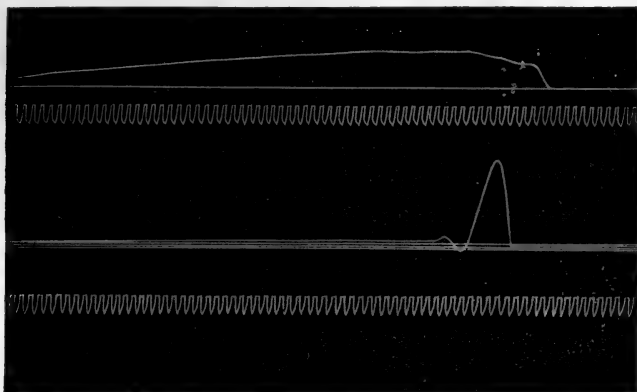


FIG. 95.—Comparison of contractions of red and white muscle of rabbit, stimulated indirectly.

Upper curve is response of the red soleus and lower curve that of the white gastrocnemius. Time marker, 50 per sec. The tracing to be read from right to left. (M.S.P.)

waiting a few minutes the muscle is stimulated by a single maximal induction-shock, and its contraction recorded. The curve shows that the response is a single slow contraction with an enormously prolonged relaxation.

Replace the hyoglossus by the gastrocnemius and sciatic prepara-

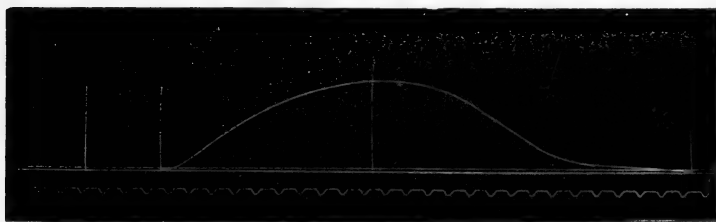


FIG. 96.—Contraction of the hyoglossus muscle.

Time marker, 100 per second. (A.P.B.)

tion and stimulate it in the same way. As soon as the first contraction is over, the muscle is stimulated again, and so on for half a dozen contractions. It will be seen that the first contraction (Fig. 97) consists of a smart initial twitch followed by a much longer contraction, and an even more prolonged relaxation. The second contraction shows the same characters to a less extent, and the

subsequent contractions become of shorter and shorter duration until they reach the normal. If the muscle be allowed to rest, the veratrine effect returns again. The absence, in the case of the hyoglossus, of the sharp initial twitch seen in the gastrocnemius contraction, is probably due to more complete poisoning of all the muscle-fibres. The gastrocnemius is more bulky, some of its fibres

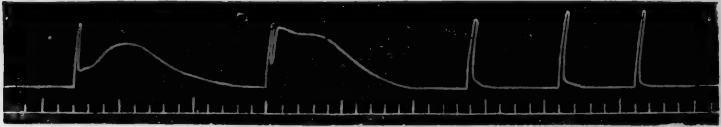


FIG. 97.—Contraction of the gastrocnemius muscle of a frog. The effect of veratrine.

The first two contractions show the characteristic effect of the drug; further stimulation produced twitches without the prolonged contraction. The curve has been reduced to one-half the actual size. The time is marked in seconds. (Pembrey and Phillips.)

remain unpoisoned and respond with a normally rapid contraction, followed by the slower and more prolonged contraction of the poisoned fibres.

Glycerine will produce a similar effect. The prolonged relaxation produced by veratrine should be compared with that seen in a muscle after repeated stimulation (see Fig. 43).

### CHAPTER III

#### STRENGTH OF STIMULUS AS AFFECTING THE EXCITATION OF NERVE AND THE CONTRACTION OF VOLUNTARY MUSCLE

In previous chapters minimal and maximal stimuli were mentioned in relation to the differences in the extent of the muscular contraction, and it might appear that the response was due to a similar gradation in the stimulation of the nerve and the contraction of the muscle. There is evidence, however, that the rule of "all or none," which has been demonstrated in the case of cardiac muscle (see p. 52) applies also to nerve and voluntary muscle. Gotch and Keith Lucas have shown that the gradation in the intensity of the electrical response of the nerve and the extent of the contraction of the voluntary muscle are due to these tissues being composed of a large number of separate fibres, whereas in cardiac muscle there is continuity between the fibres. The submaximal excitation of nerve or voluntary muscle is the maximal excitation of some only of its fibres.

The following experiment should be performed. Make a preparation of the sciatic nerve of the frog, with the nerves of the plexus and a portion of the vertebral column. Two branches of the sciatic

plexus innervate separate sets of fibres of the gastrocnemius muscle. Arrange the muscle for the record of a single twitch and make separate records of :—(1) Excitation, minimal and maximal, of the nerve trunk ; (2) similar excitation of each branch of the sciatic plexus ; and (3) simultaneous excitation of both of the branches of the sciatic plexus which supply the gastrocnemius. Compare the results.

## CHAPTER IV

### EXTENSIBILITY AND ELASTICITY OF MUSCLE WHEN AT REST AND CONTRACTED. COMPARISON WITH RUBBER

Muscle is both extensible and elastic, that is, it can be stretched beyond and will return more or less to its original length when the extending force is removed. These are important properties ; for unless muscle were readily extensible the sudden contraction of one set of muscles would in the body be liable to rupture their antagonists.

In the study of these properties a gastrocnemius preparation may be used, but a muscle whose fibres run more nearly parallel to each other is preferable, such as a sartorius preparation from a large frog.

The following experiments should be performed. The bone at the upper end of the preparation is rigidly fixed in a clamp and to the lower end is attached by a short thread or pin a brass mm. scale, having its zero at the bottom. The lower end of the scale has a small tray to carry weights or a hole by which weights can be hooked on. A pointer carried by a separate stand is placed opposite the zero of the scale. A weight of 10 grms. is attached to the scale and the amount of extension read off ; then another 10 grms. are added and so on until the load is 100 grms. or more. It will be found that the length to which the muscle is extended is not proportional to the weight used, but that, by each increase of weight, the muscle is stretched rather less, as shown in Figs. 98, 99, 101. By removing the weights one by one the elasticity of the muscle is observed ; it is not complete ; for when all the weights have been removed the muscle does not at once return to its original length. An " extension-remainder " is present, and this is the more marked the more the muscle is fatigued by the degree and duration of the extension. Therefore the observations should be made as rapidly and on as fresh a muscle as possible. It is probable that muscle in the body with its circulation intact is completely elastic.

If the muscle is replaced by a suitable piece of rubber band and the same observations are repeated on it, it will be found that the series of elongations are more nearly proportional to the weights used, thus conforming nearly to Hooke's Law, which states that the successive increments in length produced by equal increments of weight are, in

a perfectly elastic body, equal. Also, as the weights are successively removed, it will be found that the elasticity of rubber is more nearly perfect. But, if the extension be great and of long duration, an "extension-remainder" does appear and only gradually disappears.

Another method of demonstrating the same properties is to fix the upper end of a muscle-preparation in the clamp of a simple myograph and to attach its lower end to the lever by a bent pin. Attached to the lever vertically below the muscle is a scale-pan or hook to which weights can be suspended. The writing point of the lever is brought on to the surface of a stationary smoked drum and a zero line described by rotating the drum by hand. The drum is rotated back so that the point of the lever is 5 mm. from the beginning of the zero line, a weight of 10 grms. is attached to the lever, the muscle will be extended and the writing point will record a new vertical line on the drum. Turn the drum by hand so that the writing point

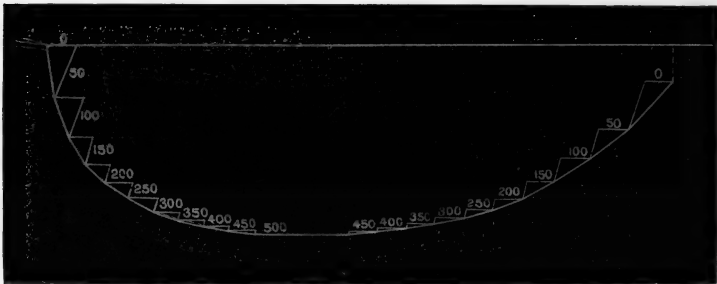


FIG. 98.—Curve of extensibility and elasticity of gastrocnemius. The figures on the curve are weights in grms. Temp., 15° C. (A.P.B.)

will describe a horizontal line 5 mm. long,<sup>1</sup> attach another 10 grms. and repeat the process until 100 grms. or more are extending the muscle. In the same way reverse the process and remove the weights of 10 grms. one by one. If now the lower ends of the vertical lines drawn by the fall and rise of the lever are joined, a curved line will be formed, showing that the extension of the muscle becomes less and less for each additional weight. Further, when all the weights have been removed, the writing point will be below the original zero line, showing an "extension-remainder" (Fig. 98). It will also be seen that the line corresponding to the elasticity of the muscle is a flatter and more gradual curve than that corresponding to the extension; this is caused by the long continued load impairing the elasticity of the muscle.

If the experiment be repeated on a piece of rubber band, the line

<sup>1</sup> By thrusting the points of a pair of fine forceps through a thin piece of cork a means of measuring off equal distances is obtained; there is a mm. scale on the induction-coil.



joining the lower ends of the vertical lines will be nearly straight, and little or no "extension-remainder" will be seen. Figs. 99, 100 show a comparison of the lines thus described for a muscle and piece of rubber loaded from 0 to 500 grms. and then gradually unloaded again.

A contracted muscle is more extensible than a resting one. This is



FIG. 99.—Elasticity curve of quiescent muscle.

To be read from right to left. The figures on the curve are for weights in grms. (M.S.P.)

of importance in the body ; for, otherwise, a sudden and powerful contraction of a muscle, trying to lift a heavy weight, would be liable to rupture either the muscle itself, or its tendon, or the bones to which it is attached. As a matter of fact, of these three structures muscle, owing to its increased extensibility during contraction, is the

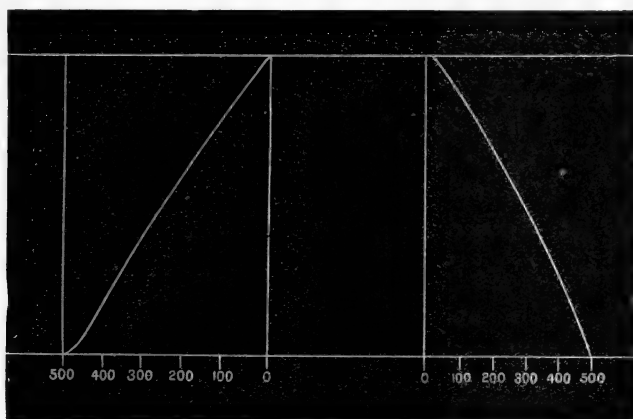


FIG. 100.—Elasticity curve of rubber tubing.

The figures represent weights in grms. (M.S.P.)

least often ruptured. In order to demonstrate this properly the muscle-preparation is attached to the clamp and lever, as in the last experiment. Arrange the apparatus for stimulating the muscle directly with single maximal induction-shocks, using a spring-key in the primary circuit. Bring the writing point on to a stationary

drum and, with the muscle weighted only by the lever, describe an abscissa line corresponding to the resting muscle. With the writing point again at the beginning of this line, stimulate the muscle once and, from the top of the ordinate so marked, draw another abscissa line corresponding to the muscle when contracted. Rotate the drum by hand, so that the writing point is now 5 mm. along the "resting" abscissa line; hang 20 grms. on to the lever and stimulate, so as to record a second ordinate 5 mm. from the first. Repeat this process, increasing the weight by an equal amount each time. In this way Fig. 101 was produced. It is clear that the distance of the lowest point of each ordinate below the "resting" abscissa line represents

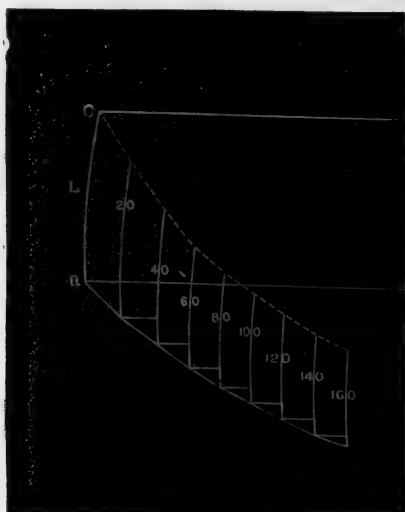


FIG. 101.—Comparative extensibility of resting and contracted gastrocnemius.

Temp., 12° C. Magnification, 5. Figures represent actual weights in grms. R is the "resting" and C the "contracted" abscissa line. (A.P.B.)

the extension of the resting muscle by a given weight, and that the distance of the top of the same ordinate below the "contracted" abscissa line represents the extension, by the same weight, of the muscle when contracted. If the lowest and then the highest points of the ordinate are joined, two curved lines are produced which represent respectively the curves of extension of resting and contracted muscle (Fig. 101). It will be seen that the extensibility of contracted muscle is absolutely greater, and increases more rapidly, than that of resting muscle. Hence, if the observations were carried far enough, the two curve lines would ultimately cross; this means that if a muscle were loaded by a weight greater than it could lift, it would during its stimulation actually lengthen

(Weber's paradox). If this were not so, we should, when trying to lift a load greater than the muscle could move, run a great risk of rupturing our muscles.

## CHAPTER V

### LOAD AND AFTER-LOAD. WORK DONE WITH INCREASING LOADS<sup>1</sup>

Muscles may be loaded in two ways: the load may be applied before the muscle has begun to contract, or only after it has already begun to contract; this latter method, in order to distinguish it from the former, is called "after-loading." Most of the muscles in the body are both loaded and after-loaded; that is, they are constantly loaded by the pull of their antagonists, and it is only after they have already begun to shorten that the main load—the weight of the limb, etc.—is applied to them. The deltoid, however, is an instance of a muscle constantly loaded by the weight of the arm; the ventricle of the heart, on the other hand, is a muscle which is only after-loaded.

The effect of load, and of its method of application on a single muscular contraction, will be studied in the following ways: (a) the contraction given by a muscle loaded and after-loaded with the same weight will be compared; (b) the muscle being just completely after-loaded, the height of contraction, with increasing loads, will be measured and the work done with each calculated.

#### **Comparison of the Contractions of a Loaded and After-loaded Muscle.**

—Arrange the apparatus for stimulating a muscle with single maximal induction shocks, using the drum as a key in the primary circuit. Fix a gastrocnemius preparation to a myograph lever, provided with an after-loading screw; by raising the screw the metal part of the lever can be supported at any level. Hang a weight of 50 grms. near the axis and raise the screw until the whole of the weight is just after-loaded; this point can be ascertained by supporting the weight with the finger, and when the muscle no

<sup>1</sup> The magnification of the movement of the muscle recorded by the lever is calculated by dividing the distance of the writing point from the axis by the distance from the axis of the point of attachment of the thread from the tendon.

The amount of actual shortening a muscle undergoes during contraction can be calculated by measuring the vertical height of the top of the curve above the base line and dividing it by the magnification.

The actual load which the muscle raises is not the whole of the weight hung near the axis of the lever, but a proportion of it, calculated by multiplying by the distance from the axis of the point of suspension of the weight and dividing by the distance from the axis of the point of attachment of the muscle.

longer tends to raise the lever off the after-loading screw, the muscle is unstretched by any load. Arrange the apparatus so that with the screw in this position the lever is horizontal. Record a single

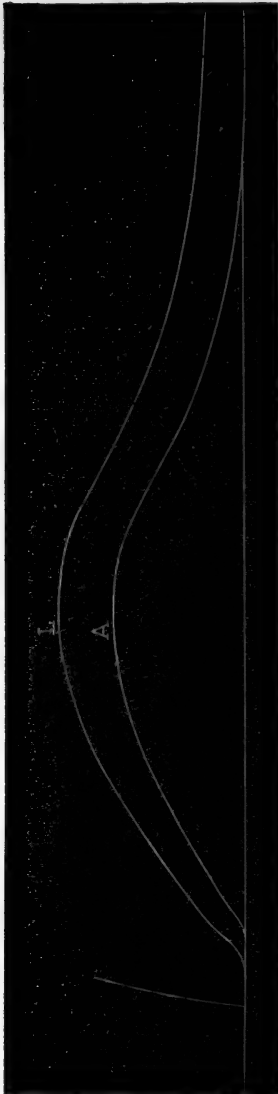


FIG. 102.—Contractions of the same muscle when loaded, L, and when after-loaded, A. Actual load on muscle was 10 grms. Temp., 10° C. Magnification, 5. (A.P.B.)

contraction of the muscle on a rapidly revolving drum, mark the point of stimulation, and draw an abscissa. Then lower the after-loading screw until the muscle is loaded with the whole weight, and superimpose on the same abscissa and with the same point of stimulation a contraction of the loaded muscle. (Fig. 102.)

The main differences between these two curves are—in the purely after-loaded muscle there is an appreciable lengthening of the latent period owing to the muscle in its unstretched condition having to take in “slack”; a diminution in the height of the contraction, owing to the absence of tension on the muscle before the contraction began. In other words, moderate initial tension increases the power of a muscle to do work.

**Relation of Load to Work done during Contraction.**—In order to record the height of contraction for a large range of weights, it is more convenient to record on a stationary drum simply the heights of a series of twitches than to superimpose a large number of curves. The apparatus is arranged for stimulating the muscle with a single maximal induction-shock, using a simple key in the primary circuit. A weight is hung near the axis of the lever of such a size that the actual load on the muscle is 50 grms.; the method of calculating this weight has been already given on p. 99. The muscle is just

completely after-loaded throughout the experiment in order to get rid of the effect of alterations in the initial tension. With the lever horizontal, the muscle is stimulated, and the height of its

contraction recorded on a stationary drum. The drum is rotated a short distance by hand ; an additional load of 50 grms. is hung from the lever, and another contraction recorded. The process is repeated until the muscle is no longer able to raise the load off the after-loading screw. Fig. 40 gives the result of such an experiment ; in it the magnification was 5, and the actual load on the muscle half of the weight hung near the axis of the lever. The following table gives in grm. mm. the work done by the muscle with the various loads :

Actual load in grm.	Actual lift in mm.	Work in grm. mm.
50	4.0	200
100	3.2	320
150	2.2	330
200	1.8	360
250	1.2	300
300	1.0	300
350	.8	280
400	.5	200
450	.4	180
500	.3	150
550	.2	110
600	.1	60
700	0	0

From the last column in this table we see that, although the height of the contractions diminishes continuously, the actual work done by the muscle increases at first rapidly and then more slowly, until it reaches its maximum with a load of 200 grms. After that point the work done begins to decrease slowly, and then more rapidly until at 700 grms. a load is reached which the muscle is unable to lift. This weight represents the " absolute contractile force " of this muscle, that is, the load which, brought to bear on the muscle at the instant of contraction, is just able to prevent it from shortening. Although the muscle is unable to lift this load, and therefore, when stimulated, does no visible mechanical work, it nevertheless liberates energy chiefly as heat.

We are now in a position to recapitulate, so far as load is concerned, the conditions necessary to obtain an optimal contraction of a muscle and to see how far they exist in the living body. Initial tension, we have seen, decreases the latent period and increases the power of the muscle to do work. In the body the muscles are constantly loaded to a slight extent, and are thus kept stretched and free from " slack." In this way movements with a short latent period, and with an absence of jerkiness, are obtained ; and the muscles by being stretched are kept irritable, awake and fit for sudden work. On the other hand, we see that a muscle, when purely after-loaded, is at a disadvantage for doing work ; yet in the body the main load is thrown on as an after-load. The advantage of this

arrangement depends upon the increased extensibility of contracting muscle; for in this way liability to rupture is reduced; further, there is a saving of energy in pulling at a dead weight through an elastic spring, instead of through an inelastic cord, since some of the energy expended would be lost in a sudden jerk, but, in the case of the spring, is stored up in it and given out again as its elastic recoil. Thus smoothness is imparted to even the most sudden movements.

## CHAPTER VI

### SUMMATION OF STIMULI

In a previous chapter the subject of summation of contractions has been dealt with. This summation of "effect" must be distinguished from the summation of stimuli, by which an inadequate stimulus, if repeated sufficiently often, becomes first adequate and then for a time increasingly effective. This is a summation of "cause," and probably plays an important part in the life of all living matter.

In order to demonstrate the summation of stimuli, arrange the apparatus for stimulating a gastrocnemius muscle directly with single induction-shocks, using a simply key in the primary circuit. Place the secondary coil at such a distance from the primary that the break-shocks are just subminimal.

Repeat the stimulus every five seconds. It will be found that sooner or later the summed excitations will cause a contraction, and, if the contractions are recorded on a slowly-revolving drum, that a well-marked "staircase" effect is produced (Fig. 103).

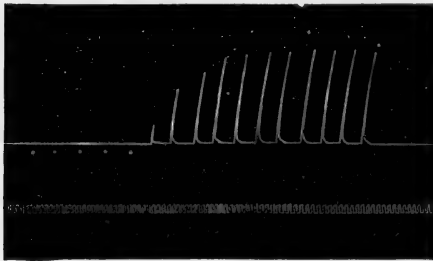


FIG. 103.—Effect of subminimal stimuli repeated every five seconds on gastrocnemius stimulated directly.

The dots mark the points at which stimuli were sent in before they became obviously effective. Time marking in seconds. (A.P.B.)

In dealing with the response of muscle to two successive stimuli, it has been seen that, when the second stimulus falls within the latent period of the first, the muscle is refractory, so far as being

able to respond with a second contraction is concerned; but it is not true that a muscle during its refractory period always entirely ignores a second stimulus.

In order to investigate this point, the apparatus is arranged as in demonstrating the effect of two successive stimuli (p. 48). The two "strikers" are placed at such an angular distance apart that the

second stimulus falls well within the latent period of the first; the muscle is stimulated directly. The secondary coil is placed at such a distance from the primary that when, by rotating the drum by hand, one of the strikers is made to pass over the naked wire, a minimal or submaximal break, but no make contraction, is obtained. A tuning fork is arranged to write under the myograph-lever, the drum is allowed to make one revolution at a rapid rate, a base line is drawn, and the points of stimulation corresponding to each "striker" are marked. Swing the lever away from the drum, but do not alter the position of the base of the stand carrying the myograph. The single contraction so recorded is the response of the muscle to two break shocks. In order to determine whether the muscle has been in any way influenced by the second stimulus, raise the second "striker," so that it will no longer touch the naked wire, and record the contraction due to the first stimulus alone (Fig. 104). It will be found that the contraction in response to the single stimulus is not so great as that due to the two stimuli. In other words, there has been a summation of stimuli during the refractory period. In the same way subminimal stimuli can be summated, but two maximal stimuli are summated only when they follow each other after an interval of less than  $\frac{1}{500}$ th second.

As has been pointed out on

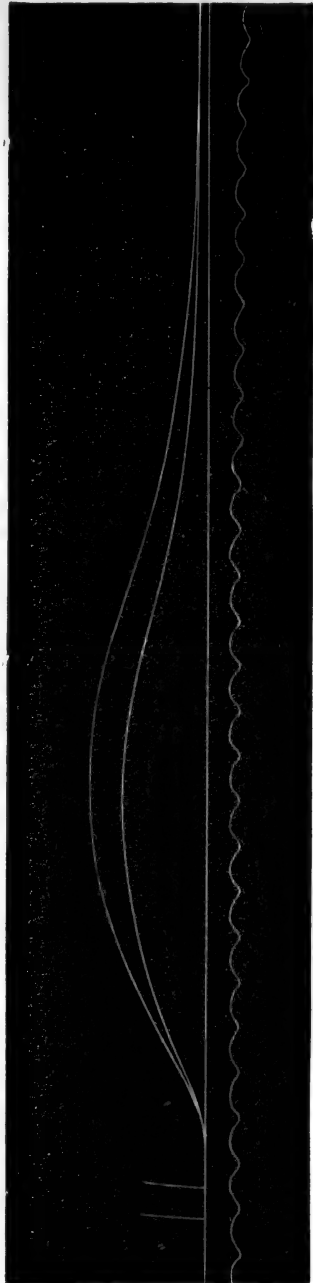


Fig. 104.—Effect of two submaximal stimuli on gastrocnemius, the second stimulus falling well within the latent period of the first.

Upper curve represents the combined effect of both stimuli, the lower curve the effect of the first stimulus alone. Time marker, 100 per sec. (A.P.B.)

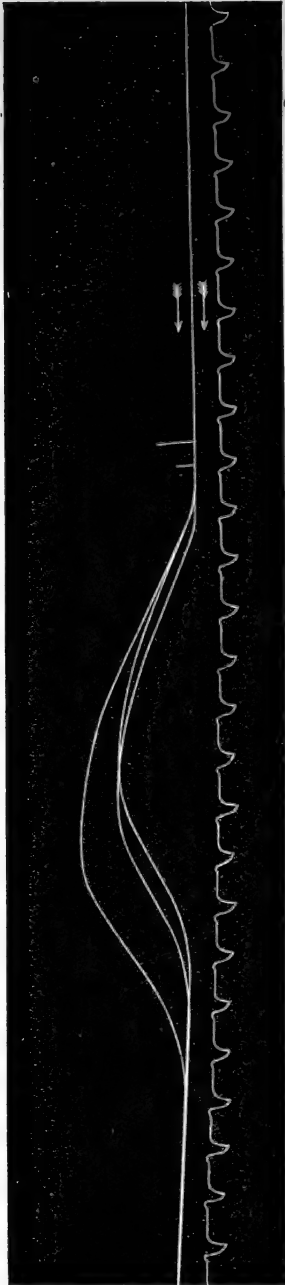


FIG. 105.—The effect of two stimuli upon the gastrocnemius muscle.

The vertical lines show the moments of stimulation; the arrows indicate that the induction shocks were in the same direction. The uppermost curve represents the combined effect of both stimuli; the other curves the effect of the separate stimuli. Time marker, 100 per second. The curves, which were taken with the Pendulum Myograph, should be read from right to left. (M.S.F.)

p. 84, when a "striker" passes over the naked wire, there is both a make and break of the primary circuit; consequently in these experiments the muscle really receives four induction-shocks, of which, according to the position of the secondary coil, all four might be individually subminimal, or the two break-shocks might be alone effective, or all four might be effective. In order to deal with the summation of two break-shocks alone, it is usual to perform these experiments with a special piece of apparatus, the spring-myograph or the pendulum myograph.

## CHAPTER VII

### EFFECT OF DISTILLED WATER AND OF VARIOUS SALTS ON MUSCLE

The various tissues of the body are all bathed in the same fluid, the lymph, which so far as the water and salts it contains are concerned, has a uniform composition. The tissues, although immersed in the same fluid, show different and characteristic properties owing to their difference in structure and chemical composition. If, however, the composition of the fluid, in which any given tissue is immersed, be altered, the composition and consequently the properties of its protoplasm must also be altered. The first effect on living matter of such a change is to cause its stimulation, and



then if the change be sufficiently profound and long-continued to produce its death.

Only two changes in the tissue fluids will be considered here, namely—(a) Gross change in the osmotic pressure of the fluid, by using distilled water or a strong saline solution ; and (b) Change in the ions in solution without alteration in the osmotic pressure of the fluid, by using solutions of various salts isotonic with frog's blood-plasma.

**Effect of Distilled Water.**—Dissect out a gastrocnemius muscle and place it, without a "trouser" of skin, in a watch-glass containing distilled water. For a few minutes the muscle may show irregular contractions, then it becomes opaque, swollen and incapable of responding to a stimulus with a contraction. The muscle is said to have passed into a condition of "water-rigor." Test the muscle with induction shocks and demonstrate that it will no longer contract.

By placing the muscle into distilled water two effects are produced—the inorganic salts in the muscle diffuse out into the water, and water is attracted by osmosis into the muscle so that each fibre becomes greatly distended with fluid. The first effect of these changes is to produce stimulation, but, as the muscle fibres are distended with fluid, they become incapable of contracting, and finally there are not enough salts left in the muscle to keep the globulins in solution ; hence the muscle becomes gradually opaque and dies.

**Effect of Strong Saline Solutions.**—This effect will be exactly the opposite of that due to distilled water ; for water will be abstracted from the tissue, and large quantities of the salt will diffuse into the muscle.

The effect on a tissue of mere abstraction of water from it is best seen by allowing a nerve to dry. Make a gastrocnemius and sciatic preparation, keep the muscle and lower half of the nerve just moist with tap-water saline, but allow the upper half of the nerve to dry. As the nerve begins to dry, irregular contractions of the muscle come on which are stopped by moistening the nerve ; showing that loss of water acts as a stimulus to nerve. If the drying is allowed to continue, the dry portion loses its irritability and dies.

Now place upon the muscle a few crystals of NaCl ; irregular contractions will soon appear. These are partly due to the abstraction of water, but also, as we shall see in the next experiments, to the stimulatory effect of NaCl.

The above experiments show that, in order to keep muscles and nerves irritable and in good condition, they must be moistened with a fluid which will neither give up nor abstract water from the tissue, i.e. which is isotonic with the animal's lymph. For this purpose a .7 per cent. solution of NaCl in distilled water has frequently been used. This solution, although isotonic with frog's blood, does not contain the calcium and potassium salts found in blood-plasma and

lymph ; and the question arises whether this alteration of the ions in solution affects in any way the properties of muscle.

In order to investigate this point, prepare two sartorius preparations with their bony attachments and without injury to their muscular fibres. Place one muscle in Biedermann's solution (.5 grms. NaCl, .2 grms.  $\text{Na}_2\text{HPO}_4$ , 2.04 grms.  $\text{Na}_2\text{CO}_3$  in 100 c.c. distilled water), and the other in .7 per cent. NaCl in distilled water.

The muscle in Biedermann's solution, especially if the solution be cool ( $3^\circ$ – $10^\circ$  C.), will after a shorter or longer interval begin to show fibrillary twitches and may even contract regularly and rhythmically as a whole. As soon as the result has been obtained, transfer the muscle to a solution made by adding to 100 c.c. of .7 per cent. NaCl solution in distilled water, 10 c.c. of a saturated solution of  $\text{CaSO}_4$ , or of a 10 per cent. solution of  $\text{CaCl}_2$  in distilled water. The spontaneous contractions will soon cease.

The other muscle placed in the pure NaCl solution may remain quiescent ; very often it will show fibrillary twitchings and irregular contractions, which are rapidly stopped by transferring the muscle to the solution containing a calcium salt as well as NaCl. Should the muscle, however, remain perfectly quiescent,<sup>1</sup> it can still be shown that it is no longer in a perfectly normal condition. After it has remained in the solution for half an hour, remove it and connect it to a myograph lever and stimulate it with a single maximal break shock. The contraction recorded on the drum will be no longer an ordinary single contraction, but a series of tetanic twitches of abnormal height and duration. Now remove the muscle, immerse it for ten minutes in the solution containing the added calcium salt, and again record its response to the same stimulus. A normal single contraction will be obtained. It is clear that sodium salts, when acting alone on skeletal muscle, have a powerful stimulatory effect, and that this can be neutralised by adding a certain proportion of calcium salt. For this reason "normal" saline solution is always made with tap-water instead of with distilled water. Some tap-waters, however, do not contain nearly enough calcium to bring about complete neutralisation of the sodium salt.

From the above experiments we learn certain facts of considerable practical importance. We see that tissues are greatly affected by changes in the osmotic pressure of the fluid surrounding them. Care must therefore be taken not to expose the tissues of an animal or man to fluids which are not isotonic with the blood-plasma. In man the solution of NaCl isotonic with the blood-plasma is only just under 1 per cent., and therefore differs widely in strength from the solution for a frog ; it is very necessary to bear this in mind when injecting fluid into veins or under the skin, and when irrigating the peritoneal cavity during operations. The saline solution tends to leak through the walls of the blood vessels ; to prevent this

<sup>1</sup> Frog's muscle differs somewhat in its behaviour in any given solution according to the time of year, there being a marked difference between muscle in the autumn and spring.

Bayliss introduced the use of a solution of gum. We further see that, when isotonic solutions of electrolytes are used, the tissues are by no means indifferent to the ions in solution. A really "normal" saline solution would, therefore, be one which contained the same salts in the same proportion as the animal's own blood-plasma. Ringer's<sup>1</sup> fluid is an attempt to make such a solution for the frog.

In all the above experiments it has been found that skeletal muscle responds to the abnormal constant stimulus by an activity which is not constant, but intermittent or rhythmical. This raises the question whether the rhythmical contraction of the heart may not be the normal response of that particular kind of muscle to the constant chemical stimulus of the blood-plasma, and the same might be also partly true of the rhythmical activity of the respiratory and vasomotor centres.

## CHAPTER VIII

### FATIGUE OF A VOLUNTARY MOVEMENT AND OF A MUSCLE-NERVE PREPARATION WITH ITS CIRCULATION INTACT

When a voluntary movement is repeated sufficiently often fatigue is produced. The seat of this fatigue has to be investigated; it might be in some part of a neurone in the central nervous system, or in some part of the peripheral nerve and muscle: in other words, the fatigue might be primarily central or peripheral. As the result of certain ergographic experiments it has been answered that this fatigue is of central origin. The experiments consisted in lifting a heavy weight suspended over a pulley by flexing a finger and registering the height of each successive lift. When the movement had been repeated until the muscle was no longer able to lift the weight at all, it was found that electrical stimulation of either the nerve supplying the muscle or of the muscle itself caused the weight to be again lifted, but to a less height than before. When the electrical stimulation had in turn fatigued the movement it was found that a voluntary contraction of the muscle was again able to lift the weight, owing, it was supposed, to the resting of the cells in the central nervous system. From these experiments it was argued that the fatigue of a voluntary movement is purely central.

The methods used in the above experiments are open to grave objections, and it is necessary to touch upon some of these in order to avoid them. The use of a heavy weight is open to the objection that the muscle, when no longer able to lift that weight, is still capable of contracting, and could well lift a lighter weight; therefore, it is better to make the muscle bend or pull on a spring, which will enable the feeblest as well as the strongest pull exerted by the muscle to be

<sup>1</sup> A modified Ringer's solution contains NaCl .7 per cent., CaCl<sub>2</sub> .0026 per cent., and KCl .035 per cent.

recorded. Again, electrical stimulation of a nerve or a muscle can be a much more powerful stimulus than that resulting from the maximal discharge of a motor nerve-cell ; consequently the fact that peripheral stimulation can make the muscle again lift the weight after voluntary impulses fail, is no proof that the fatigue was central. Further, when a nerve or muscle is stimulated by electrodes placed upon the skin, it is impossible to produce equal stimulation of all fibres. When the muscle appears to be fatigued by peripheral stimulation, then a return to volitional stimulation, by producing equal stimulation of every fibre, leads to an apparent recovery of voluntary power. In this way is to be explained the apparent paradox, that a muscle fatigued by either voluntary or peripheral stimulation shows a recovery of power when stimulated in the opposite way.

In order to investigate this subject we shall compare the curve of voluntary fatigue taken with a spring ergograph from the human abductor indicis, with the curve obtained from the frog's gastrocnemius, with its circulation intact and stimulated through the sciatic nerve.

**Porter's Spring Ergograph.**—A simple form of this instrument is shown in Fig. 44 to consist of a rigid upright iron bar which is clamped to the table. From the upper end of this projects a horizontal straight steel spring, the free end of which carries an ordinary writing point. The spring carries on its under side a short vertical steel arm, the lower end of which fits over the distal end of the second phalanx of the index finger. When the abductor indicis contracts the spring is pushed up ; by sliding the vertical arm along the spring the magnification of the movement and the strength of the spring can be altered. The hand is placed along the vertical side of the wooden support and the three outer fingers tied to it, leaving the thumb and index finger free. The forearm should be fixed to the bench in some form of support, but care must be taken not to tie down the arm sufficiently tightly to interfere with its circulation.

The subject of the experiment should sit comfortably and with his eyes shut, should not be spoken to nor in any way have his attention diverted, but should confine himself to giving a maximal contraction of his muscle every time he hears the beat of a metronome, which is set to give a beat every second. The observer takes the time of the experiment in minutes and so calculates the number of contractions recorded ; further, he has to see that the vertical arm does not slip out of position along the finger. In this way take 300 to 600 contractions on a drum revolving at an extremely low rate (Fig. 106).

At first sight the most striking feature of the curve is the more or less rhythmical waxing and waning in the height of the contractions ; this seems to be purely central in origin and to be due to variations in the strength of the voluntary impulse communicated to the muscle. Practice to a large extent does away with this rhythm. When the

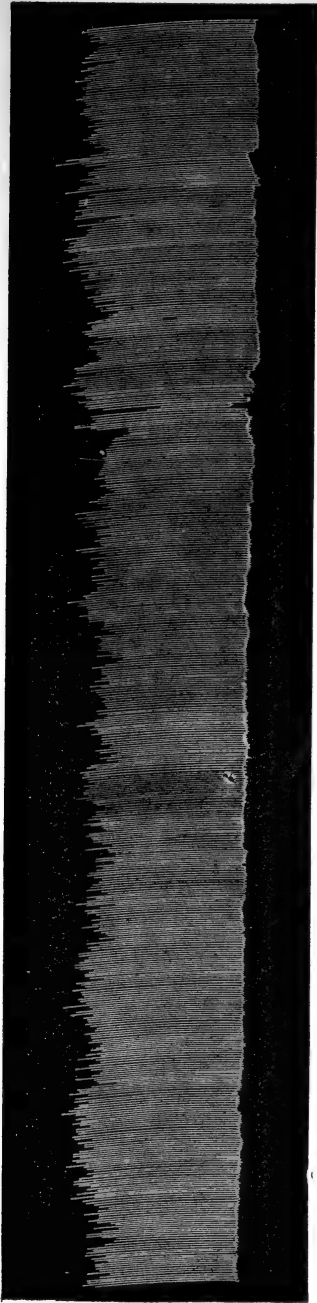


Fig. 106.—Ergographic tracing of abductor indicis taken with a spring ergograph. The tracing is to be read from right to left and represents 600 contractions performed at the rate of one per second. (A.P.B.)

height of the contraction is measured it will be found that the average height decreases during the first 180 contractions and then attains a fairly constant level, which represents about 85 per cent. of the height of the original contractions. The initial decrease is better marked in Fig. 107, and here the fatigue-level was only about 45 per cent.

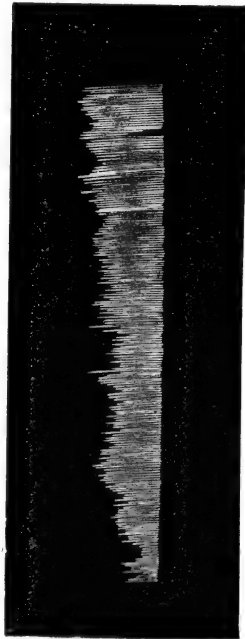


Fig. 107.—Ergographic tracing of abductor indicis of an ill-nourished boy.

The tracing is to be read from right to left and represents 150 contractions performed at the rate of one every 2 seconds. (A.P.B.)

of the original height. The characteristics of an ergographic fatigue-curve, therefore, are an initial fall which takes place during a variable number of contractions, and the attainment of a fairly constant level, which represents varying percentages of the height of the original contractions. This curve strongly suggests that during a series of

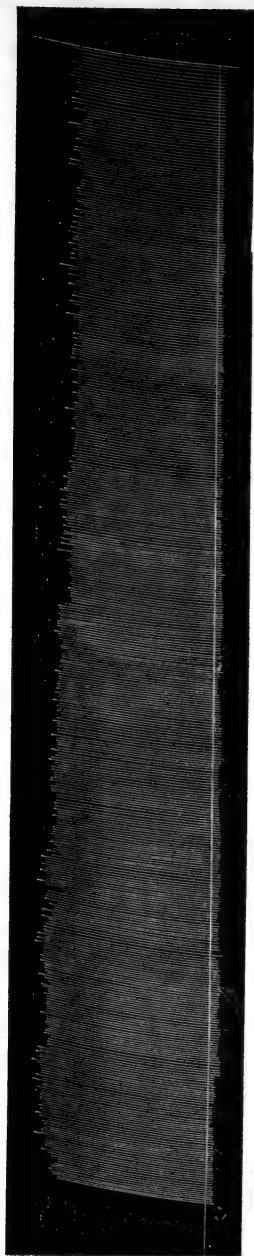


FIG. 108.—Exhaustion curve of gastrocnemius with its circulation intact.

Muscle was just after-loaded and was stimulated indirectly once every 5 seconds. This part of curve extended over a period of 45 minutes and was broken off temporarily owing to the frog moving. (M.S.P.)



FIG. 109.—Continuation of same experiment, after the leg had been cut off.

The whole curve represents a period of about 40 minutes. (M.S.P.)

contractions two processes are at work : one by which available combustible material is being used up and the products of katabolism are accumulating, and the other by which both these defects are made good by the circulation. During the early part of the

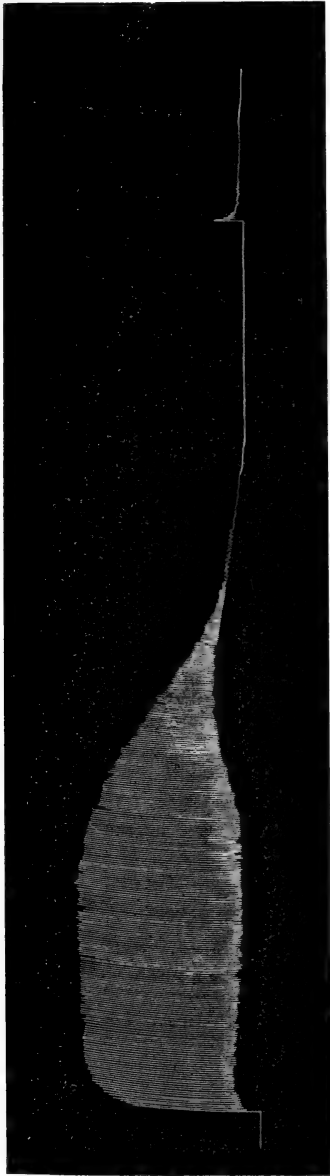


FIG. 110.—Exhaustion curve of excised and loaded gastrocnemius. Muscle was stimulated with a maximal shock every 5 secs. Exhaustion was complete at the end of 15 minutes. A slight recovery curve is shown at the end of 6 minutes' rest. (M.S.P.)

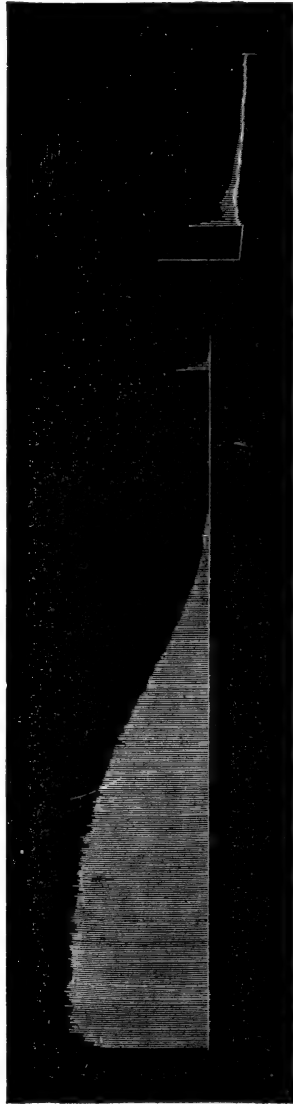


FIG. 111.—Exhaustion curve of gastrocnemius just after-loaded and stimulated once every 5 seconds indirectly. First part of curve represents 15 minutes' rest, and a short recovery curve. After another 7 minutes' rest a further recovery curve is shown with the muscle loaded instead of after-loaded. (M.S.P.)

curve the first process preponderates over the second and the height of the contraction decreases, but as soon as the two processes exactly balance each other a uniform level is maintained for hundreds of contractions. The probable seat of these processes will be referred to after the next experiment has been performed.

In order to obtain a record of the contractions of the gastrocnemius with its circulation intact, arrange the apparatus for stimulating the sciatic nerve with maximal induction shocks, using a simple key in the primary circuit. The cerebrum of the frog must be destroyed and the muscle-nerve preparation made without causing bleeding. The cerebral hemispheres are destroyed by compression, leaving the medulla and spinal cord intact, and the gastrocnemius is prepared in the usual way. A string ligature is placed beneath the gastrocnemius and tied tightly round the upper part of the tibio-fibula and the remaining muscles; the leg is then cut through below the ligature. The whole frog is placed belly downwards on the myograph-board, a strong pin is pushed through the lower end of the femur and driven firmly into the cork. A piece of moistened flannel is then pinned down over the trunk to prevent the contractions of the muscles of the trunk from disturbing the lever connected with the gastrocnemius. The skin over the middle of the thigh is divided longitudinally for a short distance, the muscles carefully separated and the sciatic nerve exposed and freed; the nerve is gently raised by slipping a thread beneath it, and the electrodes, insulated from the underlying muscles by a small piece of cork, are placed beneath the nerve. It is essential that the nerve should not be injured and should be kept properly moistened throughout the experiment. The muscle is suitably weighted and just after-loaded. The nerve is stimulated by a maximal shock every five seconds, and the contractions recorded on a drum revolving at the slowest possible rate (Fig. 108). It will be seen that the height of the contractions, although increasing at first, gradually falls off until at the end of about 200 contractions it reaches a uniform level, which represents about 85 per cent. of the original height and was then maintained with scarcely any alteration for three-quarters of an hour. This curve, therefore, is identical in general form with that obtained by the ergograph. We see at first an increase, then a fall, and then a constant level of contraction, representing probably the equilibrium between two opposite processes, which must in this case be affecting some part of the peripheral nerve and muscle. The actual seat of this peripheral change is not absolutely certain (see further Experiments in Chapter XVII).

Now cut through the leg in the middle of the thigh, so as to destroy the circulation through the gastrocnemius and continue the stimulation (Fig. 109). It will be seen that the height of the contractions rapidly and continuously decreases, and that at the end of about 320 contractions the muscle is no longer able to lift the lever off the after-loading screw.



## CHAPTER IX

## THE RATE OF TRANSMISSION OF A NERVOUS IMPULSE

The rate at which an impulse is transmitted along a nerve is important because it throws some light upon the nature of the impulse. It travels much more slowly than an ordinary electric current, and, although it is accompanied by an electric change, it is something more complex. Its rate of propagation is 27 metres per second ( $88\frac{1}{2}$  feet per sec.) in the frog's sciatic nerve, and 120 metres per second in the motor nerves of man.

(a) **In the Motor Nerves of the Frog.**—See Part I, p. 53.

(b) **In the Motor Nerves of Man.**—The velocity of the transmission of a nervous impulse in the motor nerves of man can be determined in the following way: A thick-walled india-rubber ball, similar to that used with a photographic "shutter," is connected with a recording tambour. Two clinical electrodes are moistened with strong saline solution in order to improve their conduction and contact with the skin; the large flat electrode is fastened to the leg of the subject, and the small electrode placed above the clavicle will be pressed over the brachial nerves. These electrodes are connected with the secondary coil of an inductorium, and in the primary circuit is interposed the "striker" key.

The india-rubber ball is held between the middle finger and the thumb, and the contraction of the flexor muscles will be recorded by the lever of the tambour, when the nerve is excited. The moment of stimulation is determined in the usual way (p. 42), and then the experiment is again performed, but with the small electrode pressed over the median nerve at the bend of the elbow. The moment of stimulation is again determined, in order to show that the resting position of the point of the lever has not been changed. The difference between the latency in the two contractions is measured by a tuning fork vibrating 100 times per second, and the length of nerve between the two points of stimulation is estimated; from these data the rate of transmission of the nervous impulse can be calculated.

## CHAPTER X

## THE POLARISATION OF ELECTRODES AND UNPOLARISABLE ELECTRODES

**Polarisation of Electrodes.**—Ordinary metal electrodes in contact with a muscle or nerve will be surrounded by lymph, and in this fluid electrolysis will take place during the passage of an electric current. The ions resulting from this electrolysis will be positive and negative respectively; if, therefore, the circuit of this seat of

chemical and electrical change be suddenly made or broken, a shock will be produced, for the wires of the electrodes surrounded by the electrolysed fluid will form a minute battery. This can be demonstrated by the following experiment:—A pair of electrodes, connected with a Du Bois key, is placed under the sciatic nerve, which has been exposed in the thigh of a pithed frog. Making or breaking the circuit causes no contraction. The two wires of a Daniell battery are connected with each side of the Du Bois key, and the current is allowed to pass through the nerve for several seconds. Then these two wires are rapidly disconnected from the battery and key; the key is closed and opened, and each time a contraction of the muscles of the leg is caused. This make and break can be repeated several times with a similar result, until the polarisation has disappeared.

This experiment shows the necessity of unpolarisable electrodes in experiments upon the effects produced in nerve and muscle by the passage of a constant electric current, and also the necessity of using a Du Bois key as a bridge to short-circuit the electrodes.

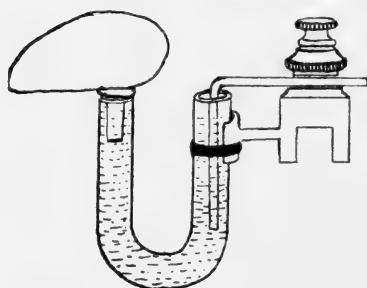


FIG. 112.—Unpolarisable electrode.  
Burdon-Sanderson's pattern.

unpolarisable electrodes are used. The electric current from the battery is conducted through media which are not liable to polarisation.

The structure of Burdon-Sanderson's electrodes is shown in the following diagram (Fig. 112). A smooth amalgamated zinc rod dips into a saturated solution of zinc sulphate, which in turn conducts the current by means of a plug of kaolin or china clay, made into a thick paste with normal saline solution (.75 per cent. sodium chloride). The plug rests upon a small glass tube with a flange; this delays the spread of the zinc sulphate into the kaolin. The nerve or muscle can be placed in contact with the plug of kaolin, or may be connected thereto by threads saturated with normal saline solution and kaolin. The plug must be kept moist with normal saline solution, for the electrodes have a high resistance.

The electrodes must be set up with clean hands and material, otherwise polarisation will occur. The solution of zinc sulphate must not be allowed to touch the tissue, for chemical excitation would occur. Kaolin and normal saline solution do not stimulate muscle and nerve.

**Unpolarisable Electrodes.**—  
The preceding experiment has shown that the electrolysis occurring around the ordinary metal electrodes may easily act as an exciting electric current, and thus cause errors in experiments. In order to avoid this

The previous experiment on the polarisation of electrodes should be repeated with the unpolarisable electrodes. The result will be negative if the electrodes have been well and truly made.

CHAPTER XI

TRANSMISSION OF A NERVOUS IMPULSE IN BOTH DIRECTIONS

The excitatory state produced by stimulation of a nerve can be transmitted in both directions. This can be shown by the following experiments.

**Sartorius Experiment.**—The sartorius muscle lies on the ventral surface of the thigh (Fig. 31) and its outlines can be made distinct by sponging it with the frog's heart full of blood. The muscle is dissected out and its iliac end is divided into two portions (Fig. 113). Stimulation with a weak induction shock at (*a*) or (*a'*), when there are no nerve-fibres, will produce a contraction of the one half of the muscle. Excitation, however, at (*b*) or (*b'*), where there are nerves, will evoke a contraction of both halves.



FIG. 113.—Diagram of the sartorius experiment to show the transmission of a nervous impulse in both directions.

**Gracilis Experiment.**—The gracilis muscle of the frog is in two portions completely separated by a tendinous intersection (Figs. 31, 114). Both halves of the muscle are supplied by a single nerve, the



FIG. 114.—Diagram of the gracilis experiment to show the transmission of a nervous impulse in both directions.

individual fibres of which divide and supply both halves of the muscle. Stimulation of any kind at (*a*) or (*a'*), where there are no nerve-fibres, causes only the corresponding half of the muscle to contract; but excitation at (*b*) or (*b'*), where the nerves lie, will cause both halves to contract.

## CHAPTER XII

## THE RELATION BETWEEN MUSCLE AND NERVE. THE INDEPENDENT EXCITABILITY OF MUSCLE

In addition to the experiments which have been described in the elementary course (page 33), the following experiment upon the sartorius muscle should be performed.



FIG. 115.—  
Diagram of the sartorius muscle to show the distribution of its nerves.

The muscle is carefully dissected out and will contract when its nerve, which passes into the muscle at the middle of its inner border, is cut across by the scissors. If the muscle be placed between two glass-slides and examined under a microscope, the distribution of its nerve can be seen to resemble that shown in the diagram (Fig. 115). The finer branches of the nerves and even the end-plates can be more readily seen if the muscle be treated with acetic acid. There are no nerves in the terminal portions of this muscle, which consists of fibres running in a direction parallel with its length.

The sartorius muscle is dissected from the other thigh and the nerveless parts are stimulated by a pinch with a pair of forceps or by an electrical shock; they contract, the muscle possesses independent excitability.

The absence of nerves from the terminal portions can also be shown in the following way.

The muscle is suspended from its tibial end and is lowered until the cut iliac end touches some strong glycerine contained in a watch-glass; it does not contract. A thin transverse slice is cut away and the muscle is again lowered into contact with the glycerine; there is still no contraction. This procedure is repeated until the nerves are cut across and on contact with the glycerine are stimulated and make the muscle pass into a contracted condition.

## CHAPTER XIII

## THE ELECTROMOTIVE PROPERTIES OF MUSCLE AND NERVE

In uninjured and resting muscle and nerve there is no electric current, but during activity a current, the "*current of action*," is produced. Injury causes local activity around the damaged tissue, and is therefore accompanied by an electric current, the so-called "*demarkation or injury-current*." This electrical current produced

by injury is, as Gotch pointed out, to be considered as a *current of action*. These facts can be demonstrated by the following experiments.

**The Rheoscopic Frog. Galvani's Experiment, Contraction without Metals.**—A long length of the sciatic nerve is dissected in a pithed frog and the muscles of the thigh are exposed and cut across. The trunk of the sciatic nerve is laid along the longitudinal surface of the muscles of the thigh, and then by raising the end of the nerve by a small glass rod the transverse section of the nerve is allowed to fall upon the cut surface of the muscles (Fig. 116). At this moment a twitch of the muscles of the leg moves the foot or toes. The circuit of the electric current in the muscle has been completed through the nerve. The section of the muscle-fibres has produced a local contraction of the fibres, and this is accompanied by an electrical change which is sufficient to produce excitation when it is passed through an excitable nerve.



FIG. 116.—Diagram of Galvani's experiment. Contraction without metals.

**Secondary Contraction or Secondary Twitch.**—Two muscle- and nerve-preparations are made; the nerve of A is so placed upon the muscle B that the cut surface of the nerve lies upon the tendon and its longitudinal surface upon the muscle-fibres (Fig. 117). The nerve of preparation B is stimulated by a weak induction-shock,

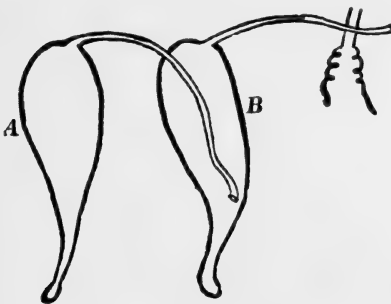


FIG. 117.—Diagram of the experiment on secondary twitch.

and thus its muscle is excited and made to contract; the muscle A will also contract. The contraction of the muscle B is accompanied by an electrical current, the "*current of action*," which passes through the nerve A and thus produces a contraction in the muscle A. This is not due to an escape of electrical current from the electrodes, for a secondary twitch can be obtained if mechanical or thermal stimuli be used to

excite the nerve of preparation B. Further, ligation of the nerve B with a moist thread will show that there is no escape with a weak induction-shock; the ligature destroys the physiological continuity and prevents the passage of the excitatory state but not that of an electrical current.

**Secondary Tetanus.**—If the nerve be stimulated with a rapid series of induction-shocks the muscle B goes into tetanus and its “*currents of action*” stimulate the nerve A, with the result that the tetanus is also observed in the muscle A. This “secondary tetanus” can be produced by rapid mechanical stimuli.

**Secondary Twitch from the Heart.**—If a freshly prepared and very excitable nerve be laid upon the heart of a frog,<sup>1</sup> so that the cut end of the nerve is on the base and the longitudinal surface upon the apex of the ventricle, a twitch of the muscle connected with the nerve is observed at each contraction of the ventricle. Each time the muscle-fibres of the ventricle contract, a “*current of action*” is produced and stimulates the nerve.

A fine glass rod should be placed under the middle portion of the length of nerve, which lies on the ventricle, so that the current may not be short circuited.



FIG. 118.—Diagram of the experiment to show the stimulation of a muscle by the “*current of action*” of another muscle.

**Stimulation of a Muscle by the “*Current of Action*” of another Muscle.**—The sartorius muscle is very carefully dissected on each side, and then the one muscle is placed overlapping the other; the contact of the two muscles is secured by gentle pressure with two pieces of cork (Fig. 118). Stimulation of one muscle will produce a contraction in both; the “*current of action*” in the first stimulates the second muscle.

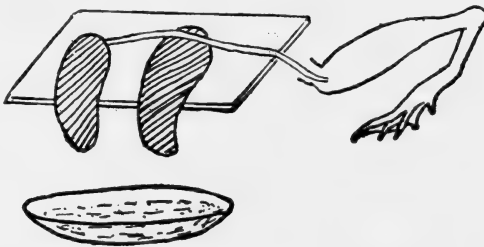


FIG. 119.—Diagram of the experiment to show the stimulation of a nerve by its own “*current of injury*.”

**Stimulation of a Nerve by its own “*Current of Injury*.”**—Two plugs of kaolin moistened with normal saline solution are placed upon a piece of glass, and the tails of the plugs are made to hang

<sup>1</sup> For these preparations the frogs should have been kept cold for some time before the experiment.

over the edge (Fig. 119). The sciatic nerve of a pithed frog<sup>1</sup> is carefully dissected down to the knee, the thigh is cut across, but the leg and foot are left intact. The nerve is so placed that its cut surface is upon one plug and its longitudinal surface upon the other plug. A watch-glass filled with strong saline solution, which is a good conductor of electricity, is suddenly brought in contact with the ends of the kaolin plugs; thus the circuit is suddenly made and can be suddenly broken by the removal of the watch-glass. If the preparation be very excitable, a twitch is observed at each make and break of the circuit; the nerve is stimulated when the circuit of its "current of injury" is completed or broken.

## CHAPTER XIV

### THE ELECTROMOTIVE PROPERTIES OF MUSCLE AND NERVE —Continued. THE GALVANOMETER AND THE CAPILLARY ELECTROMETER

**DEMONSTRATIONS.**—The galvanometer and the capillary electrometer are delicate instruments which are easily damaged; they are employed to investigate the electromotive properties of muscle and nerve. The essential experiments upon that subject have already been performed by means of the so-called "rheoscopic frog." In this course, therefore, the experiments with the galvanometer and the capillary electrometer will be demonstrated to the student and only brief details will here be given.

The **Galvanometer** employed in these experiments is Kelvin's reflecting galvanometer. It consists of a suspended system of magnets so arranged as to make the system nearly "astatic"; the magnets are surrounded by coils of many turns of fine insulated wire. The resistance is high, from 5,000 to 20,000 ohms. The movements of the mirror attached to the magnets are indicated by a spot of light upon the scale.

The amount of current sent through the galvanometer is regulated by means of a *shunt*, which is a resistance box whereby  $\frac{1}{10}$ th,  $\frac{1}{100}$ th, or  $\frac{1}{1000}$ th of the total current can be sent through the galvanometer.

The electric current from the muscle or nerve is led off by means of unpolarisable electrodes, but before an experiment is performed the electrodes are tested, for in most cases they are not perfectly iso-electric. Any small deflection of the galvanometer due to this cause is compensated by a graduated current from a standard battery sent through the galvanometer in the opposite direction.

Perfectly uninjured muscle and nerve are iso-electric, but they are generally slightly damaged during the process of dissection and

<sup>1</sup> For these preparations the frogs should have been kept cold for some time before the experiment.

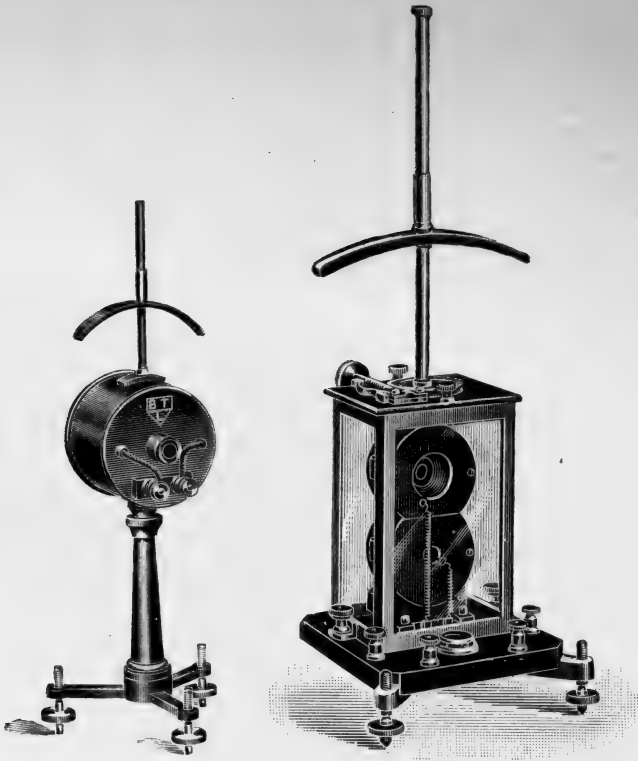


FIG. 120.—Galvanometers.

preparation. The deflection due to this **current of injury** or **demarkation current** (wrongly called the current of rest) is measured and is then increased by a more pronounced injury caused by touching one

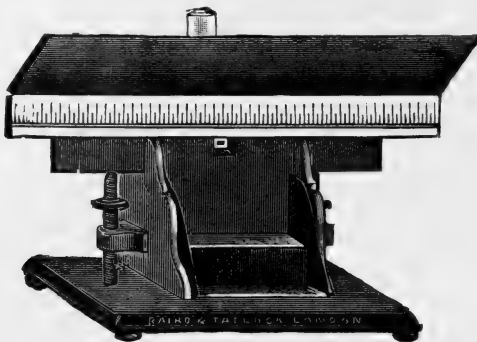


FIG. 121.—Scale and lamp for the reflecting galvanometer.



end of the muscle with a hot wire. The muscle is now stimulated by a tetanising current applied to its uninjured end ; the deflection of the galvanometer is in the reverse direction, due to the **current of action** (formerly called the negative variation) which is produced when the muscle contracts.

The current of injury is, as Gotch pointed out, to be considered as a local current of action ; around the injured portion the tissue is in a condition of excitation.

Similar experiments are demonstrated upon nerve.

**String-galvanometer—Electro-cardiograph.**—The student should read the description of this instrument in his Text-book of Physiology.

**Lippmann's Capillary Electrometer.**—This instrument is a delicate electrical manometer, and is more suitable than the galvanometer for the investigation of the electromotive properties of the frog's heart ; it responds to very rapid changes of electrical potential. It consists (Fig. 122) of a glass tube *C* drawn out at one end to a fine capillary tube ; this is filled with mercury and is connected with a pressure apparatus by the rubber tubing *RT*. The capillary tube dips into a small trough filled with 10 per cent. sulphuric acid ; the bottom of this vessel is covered with mercury *M* in order to provide good electrical conduction with the platinum wire. The movements of the column of mercury in the capillary tube are observed by means of a microscope fitted with a micrometer scale.

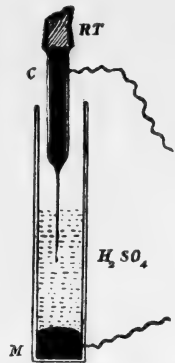


FIG. 122.—Diagram of the capillary electrometer.

The passage of an electrical current through the capillary tube alters the surface tension, and this alteration causes a movement of the mercury in the capillary tube. The movement of the column of mercury is from positive to negative, and the extent of the movement is roughly proportional to the difference in electrical potential. Based upon these facts are the determination of the direction of, and the measurement of the electromotive force of, the current which is under investigation.

With the capillary electrometer the *electromotive properties of the frog's heart* are demonstrated. The base and the apex of the ventricle are led off by unpolarisable electrodes to the electrometer : each time the heart contracts there will be a diaphasic variation, the contracted portion at first becomes negative and then positive to the uncontracted part.

## CHAPTER XV

## THE EFFECT OF A CONSTANT CURRENT UPON MUSCLE AND NERVE

Muscle and nerve consist of complex chemical substances, and contain about 70 per cent. of water in which various salts are dissolved. Moreover, they are bathed in lymph.

The passage of a constant current through a liquid produces electrolysis; thus, in the case of water, oxygen is given off at one plate, hydrogen at the other. Animal tissues, containing, in addition to a large percentage of water, salts and proteins, are also the seat of electrolysis during the passage of a constant current; the ions are probably of a complex nature. These changes in nerve and muscle are shown by alterations in excitability and conductivity.

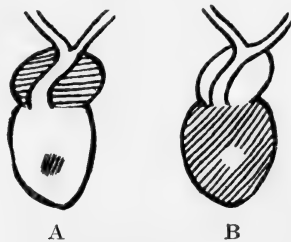


FIG. 123.—Diagram of the frog's heart to show the effects of the make and break of a constant current upon muscle.

In A the ventricle is represented as pale and contracted, with a small shaded area to represent the flushed and uncontracted portion of the ventricle; that is, a local diastole during general systole. This condition can be produced by the make of the anode or the break of the kathode of a constant current. In B the ventricle is dilated and flushed, with a small pale area of contracted muscle; that is, a local systole during general diastole. This condition can be produced by the make of the kathode or the break of the anode.

These it is necessary to consider in relation to the changes which occur at the anode and kathode during the make and break of the constant current. The simplest experiment can be made upon the frog's heart.

**The Effects of Anode and Kathode upon the Frog's Heart.**—The brain and spinal cord of a frog are pithed and then the heart is exposed. Care should be taken to avoid the severance of large blood vessels in order that the vascular system may be well filled with blood. The pericardium is opened and the heart is observed; the ventricle during systole is pale owing to the contraction of its muscle fibres forcing out the blood from its spongy walls; during diastole, when the muscle is relaxed the ventricle is flushed owing to

its distension with blood. There are no blood vessels in a frog's cardiac muscle.

The ends of two pieces of ordinary insulated wire are well cleaned and are connected with a Daniell battery ; the clean free ends of the wires are bent back so that there will be smooth surfaces to apply to the heart. The wire connected with the copper of the battery is the *anode*, that with the zinc is the *kathode*.

In the frog's mouth is placed the kathode, for there good contact is obtained with a moist conductor ; the anode is placed upon the ventricle. Now it will be found that during the systole of the ventricle that portion of the muscle which is around the anode will be flushed, uncontracted, and bulging outwards—*the anode at the make of the circuit produces a local diastole during general systole* (Fig. 123, A). The rhythmic power of the cardiac muscle around the anode is diminished, so that it remains uncontracted.

If now the wire be suddenly removed from the heart, the break of the anode causes an increased excitability of the muscle to which it had been applied, there is a local pallor ; the cardiac muscle is here contracted during the general diastole of the heart. *The break of the anode produces a local systole during a general diastole.*

The kathode is now applied to the heart and the anode is placed in the frog's mouth. There is produced a *local systole during the general diastole of the heart*. The kathode increases the excitability of the cardiac muscle, and thus the fibres affected remain contracted. The end of the wire is kept in contact with the ventricle for about a minute and is then suddenly removed ; a flushed and bulging spot will indicate the region to which the wire had been applied. *The break of the kathode produces a local diastole during general systole*, for the disappearance of the condition of katelectrotonus is accompanied by a fall in excitability.

This simple experiment shows that the make of the kathode and the break of the anode excite, that the make of the anode and the break of the kathode depress. This is also true in the case of nerve.

## CHAPTER XVI

### THE EFFECT OF A CONSTANT ELECTRICAL CURRENT UPON THE EXCITABILITY AND CONDUCTIVITY OF NERVE

The passage of a constant current produces changes in the excitability of nerve. At the anode there is a condition known as **anelectrotonus**, the excitability is diminished ; at the kathode there is an increase in excitability, a state of **katelectrotonus**. The conductivity is also affected, there is a fall in both the anodic and

kathodic regions. These effects can be shown by the following experiment.

One Daniell battery is connected by two wires with a Pohl's reverser whereby the direction of the current can be changed; from the reverser the wires pass by means of a Du Bois key to a pair of unpolarisable electrodes. This is the polarising circuit. The stimulating circuit is set up separately for the production of single induction-shocks (Fig. 124). A preparation of the sciatic nerve and gastrocnemius muscle is carefully made from a recently pithed frog, and is placed in a moist chamber; a pin is fixed through the lower extremity of the femur, and the tendo-Achillis is connected by a thread with a lever. The sciatic nerve is placed across the kaolin plugs of the unpolarisable electrodes. The drum can be moved by hand. A minimal stimulus for the nerve is obtained, care being

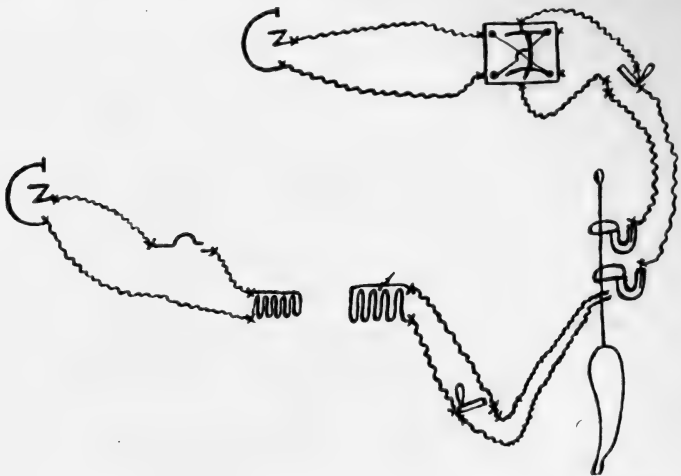


FIG. 124.—Diagram of the experiment on the effects of a constant electrical current upon the excitability and conductivity of nerve.

taken to use only the break or make-shock. The minimal contraction is recorded on the stationary drum.

The current from the polarising circuit is closed in an ascending direction, so that the current enters the nerve on the side near the muscle and immediately above the stimulating electrodes, which are connected with the inductorium. The nerve around the point of entry or anode of the polarising current is depressed in its excitability, and the application of a minimal, or even stronger, stimulus is no longer effective (Fig. 125). The polarising current is short-circuited by the Du Bois key, and by means of the reverser is changed in its direction, so that on opening the Du Bois key the current is descending, and the area of nerve near the stimulating electrodes passes into a condition of katelectrotonus. The minimal stimuli now

become maximal, owing to the increase in the excitability of the nerve at the kathode.

The above experiments can be repeated with a crystal of common

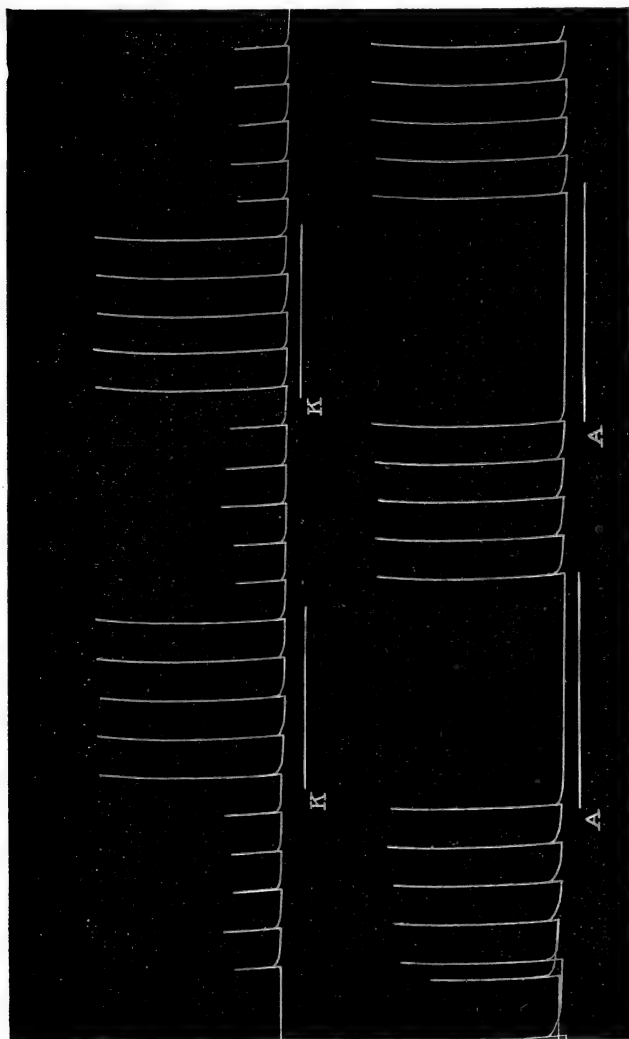


FIG. 125.—The effect of a constant electrical current upon the excitability of nerve.

(1) The nerve was stimulated at regular intervals by single induction shocks almost strong enough to produce a maximal contraction. The horizontal lines marked A show the time during which the polarising current was closed in an ascending direction, that is the duration of anelectrotonus; the single induction shocks were no longer effective.

(2) The nerve was stimulated at regular intervals by minimal induction shocks. The horizontal lines marked K show the time during which the polarising current was closed in a descending direction, that is the duration of katelectrotonus around the exciting electrodes; the minimal shocks become maximal. The curve should be read from left to right. (M.S.P.)

salt placed in the position of the stimulating electrodes. The salt causes chemical excitation, and the muscle shows incomplete tetanus, which is quelled by anelectrotonus, and augmented by katelectrotonus (Figs. 127, 128).

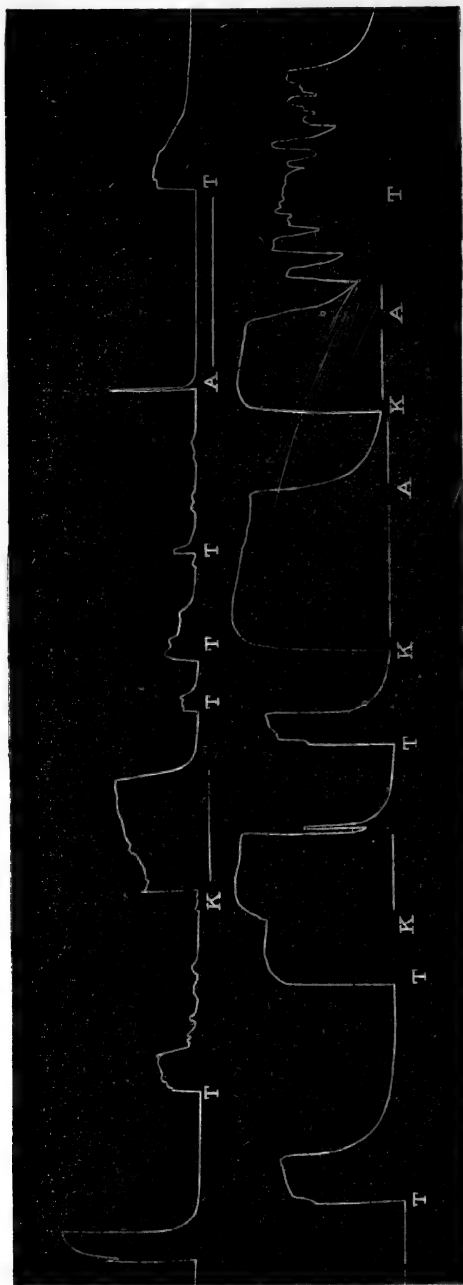


FIG. 126.—The effect of a constant electrical current upon the excitability of nerve.

The nerve was stimulated by weak tetanising shocks obtained with Helmholtz's arrangement; the incomplete tetanus T was recorded. The production of a condition of katelectrotonus, K, increased the effect of the stimuli and thus augmented the tetanus. The production of a condition of anelectrotonus, A, around the exciting electrodes abolished the effect of the stimuli; the excitability of the nerve was diminished, the tetanus ceased. The curves should be read from left to right. The tracing has been reduced in size. (M.S.P.)

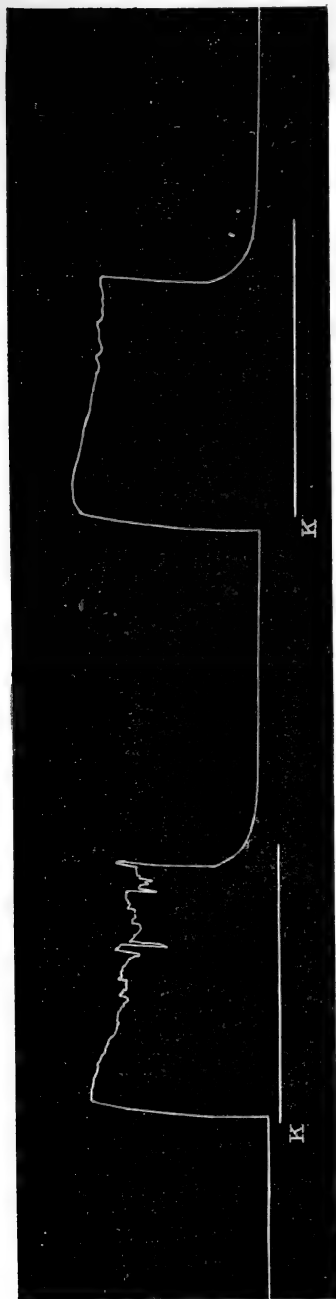


FIG. 127.—The effect of a constant electrical current upon the excitability of nerve.

A crystal of common salt was employed for the stimulation of the nerve; the chemical stimuli were subminimal, but became effective directly the nerve around the crystal of salt was thrown into a condition of katelectrotonus, K—, the chemical stimulation of the nerve caused incomplete tetanus of the muscle. The curve, which has been slightly reduced in size, should be read from left to right. (M.S.P.)

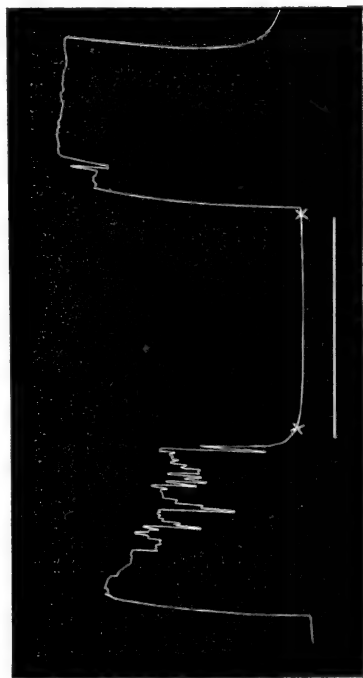


FIG. 128.—The effect of a constant electrical current upon the excitability of nerve.

This tracing is a continuation of that in Fig. 127. The chemical stimulation of the nerve from the penetration of the salt produced incomplete tetanus, which could be abolished or quelled by throwing the stimulated portion of the nerve into a condition of anelectrotonus. The beginning, duration and end of the stage of anelectrotonus are indicated by the stars and horizontal line. The curve should be read from left to right. (M.S.P.)

The conditions of anelectrotonus and of katelectrotonus spread on each side of the respective electrodes, so that between the poles there is a decrease in excitability near the anode, an increase of excitability near the kathode, and no alteration in excitability at a point about midway between the poles. These changes in the interpolar region are best studied by means of a mechanical stimulus, such as Tigerstedt's hammer, which is worked by an electromagnet. The results of such an experiment are shown in Fig 129.

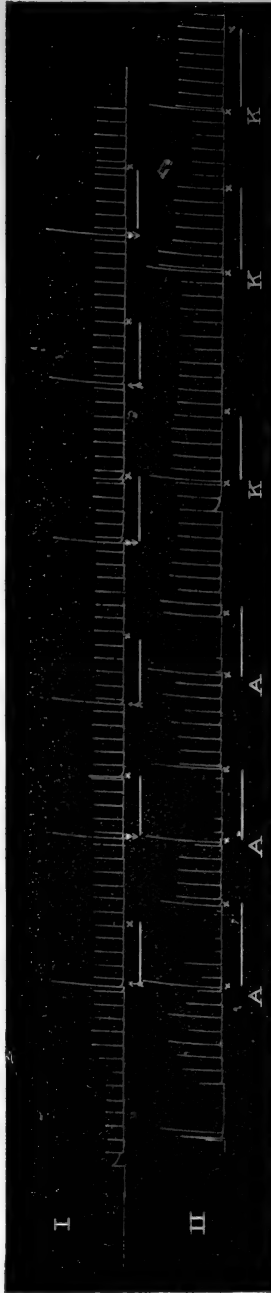


FIG. 129.—The effect of a constant electrical current upon the excitability of nerve in the interpolar region.  
 I. The stimuli produced by the vibrating hammer were applied at the *middle point* between the polarising electrodes. The moment of closure of the constant current is shown by the big contraction, its duration by the horizontal line and stars, and its direction, ascending or descending as the case may be, by arrows. The contractions are practically equal whether there is or is not a polarising current present; the excitability of the middle point is unchanged. II. The vibrating hammer was applied in the interpolar region, but near the electrode anelectrotonus A was produced, the stimuli became subminimal; then katelectrotonus K was produced, the effectiveness of the original stimuli was increased. The moment of closure of the constant current is shown by the big contraction, its duration by the horizontal line and stars. The curves, which have been reduced to one-half the original size, should be read from left to right. (M.S.P.)



The effect of the constant current upon the conductivity of the nerve is determined upon the same preparation. The stimulating electrodes are placed upon the central part of the nerve ; a minimal stimulus is found, and its effect is recorded upon the stationary drum. The polarising circuit is now closed through the nerve in either the ascending or descending direction, and then the minimal stimulus is again applied. It is no longer effective owing to the decrease in the conductivity of the nerve. This change in the conductivity of nerve is also shown in the experiment upon the absence of fatigue in a stimulated nerve (Chapter XVII).

## CHAPTER XVII

### THE ABSENCE OF FATIGUE IN A STIMULATED NERVE

Nerves are not subject to obvious fatigue, if they be repeatedly stimulated for long periods of time. The following experiment not only demonstrates this fact, but at the same time shows that the passage of a constant electrical current through a portion of a nerve blocks the transmission of the excitatory state which is produced in the nerve by a stimulus applied above the polarising electrodes (p. 124).

An induction coil is arranged for faradic shocks, and a pair of unpolarisable electrodes are connected by a Du Bois key with a Daniell cell. The two sciatic nerves of a pithed frog are dissected up to their points of exit from the vertebral column, which is then cut across above the nerves. The thighs are cut away above the knee, and the two legs with their nerves are placed in a moist chamber, and are fixed by pins pushed through the lower extremities of the femora. The stimulating electrodes, which are connected with the secondary coil by means of a Du Bois key, are placed under both sciatic nerves ; the unpolarisable electrodes are placed under one sciatic nerve midway between the muscle and the stimulating electrodes. The induction shocks are now allowed to pass through both nerves for a few seconds ; the muscles of both legs are thrown into tetanus. The stimulation is stopped and the polarising current is passed through the one sciatic nerve. The faradisation of both nerves is again commenced ; the muscle in the one case will be sent into tetanus and quickly fatigued, but the other muscle shows no contraction, for the polarising current passing through its nerve blocks the passage of the nervous impulses evoked by the stimulating electrodes. When the first muscle is fatigued the polarising current should be broken ; the block is removed from the course of the sciatic nerve of the other muscle, which is at once tetanised by the stimulation of its nerve.

## CHAPTER XVIII

## RESPIRATION

**Examination of the Chest of Man.**—Much can be learned by simple methods of examination, and it is of the greatest importance that the medical student should rely more upon his sight, hearing and touch, than upon the graphic records obtained with different forms of apparatus.

**Inspection.**—The chest of a man stripped to the waist is examined and the following points are noted: (i) The shape, whether the thorax is strongly built and symmetrical, (ii) its mobility, whether the two sides move equally. The condition of the abdominal wall should then be examined, and attention paid to the development of its muscles and the movements during respiration.

The measurement round the chest of an adult man is about 35 inches and can be taken with a tape. The increase in circumference produced by inspiration is about 2 to 3 inches. It is impossible, however, to determine by such measurements whether a man has a good "wind" or not. A well-developed chest generally means that a man has lived an active life and has a good heart and lungs, but great variations are found in the shape of the chest of healthy men. The true test of a man's heart and lungs is whether he can respond to the demands of muscular exercise without undue breathlessness and distress. Even this test must be applied with intelligence, for the man may be under-fed, and may have led a very sedentary life.

A graphic record of the shape of the chest in different planes can be obtained with the *cyrtometer* (see Part I, p. 71).

The movements of the chest and abdomen should be observed and their relationship to inspiration and expiration determined. Some subjects show marked abdominal or diaphragmatic breathing, others breathe more by the thorax. In women the movement of the upper part of the chest is greater than in men; the causes of this difference are to be ascribed to the constriction of the abdomen and lower portion of the thorax by corsets and to the greater mobility of the thorax, due to the fact that in civilised countries the women do less muscular work than the men. If hard work is frequently performed with the arms the upper portion of the thorax becomes more rigid, and this is an advantage, for it gives a better purchase for the contracting muscles.

There is no sound basis for the dogmatic teaching about thoracic and abdominal breathing of some so-called specialists in physical training. Healthy children do not need lessons in breathing, but opportunities for muscular exercise, for games in the open air. No reasonable athlete would attempt to improve his "wind" except by training it by progressively graduated runs. A good "wind" is

something more complex than a big or mobile chest ; it involves the heart which forces the blood through the lungs. Artificial breathing exercises are unsound ; healthy games and sports train the whole body, the component parts of which are mutually dependent.

At rest breathing is performed by healthy subjects with the mouth closed, but during severe work it is opened instinctively and with advantage, for there is then less resistance to the passage of the air in and out of the chest, and the loss of heat is facilitated.

The rate of respiration in healthy adult men at rest varies from about 10 to 23 per minute ; men who breathe slowly take deep breaths ; those who breathe quickly take shallow breaths.

**Palpation.**—By placing the flat of each hand upon corresponding portions of the chest it is possible to compare the movements of the two sides of the thorax. If the subject be told to speak, to say “ ninety-nine,” for example, the vibration of the voice, *vocal fremitus*, is propagated through the bronchi to the wall of the chest, and thus to the hands of the examiner.

**Percussion.**—If a tap with the finger be given to the top of a table, the note will be dull over the part directly supported by the leg, but more resonant in the middle of the table. It is also easy for most men to detect a difference in the sense of resistance when the tap is given ; it is greater with the dull note. In a similar manner the level of water in a tub can be determined. Such a method of investigation of underlying structures is known as percussion.

Firmly place the index finger of the left hand on the chest and tap it with the middle finger of the other hand. Determine the differences in note and resistance over the various parts of the thorax. On the right side the resonance extends from the apex of the lung in the supra-clavicular fossa to the beginning of the dulness produced by the liver under the 6th rib. On the left side it extends to the cardiac dulness which begins at the 4th rib.

Make the subject take a deep breath, and then by percussion on the right side demonstrate that the limit of resonance is increased owing to the expansion of the lungs.

**Auscultation.**—The respiratory and cardiac sounds can be heard by placing the ear against the chest, or by means of a wooden or binaural stethoscope. Over the trachea, or at the level of the 7th cervical spine, the harsh blowing sounds, due to inspiration and expiration, are heard ; these “ bronchial sounds ” are produced by the vibration of the air at the orifices of the vocal cords and divisions of the trachea and bronchi.

Another sound, the “ vesicular murmur,” is heard on listening to parts of the chest wall where the lung is in contact. It is a soft breezy sound which increases during inspiration and dies away during the first third of expiration. There are several views about the causation of this sound ; it may be due to conduction of the bronchial sounds.

## CHAPTER XIX

## INTRA-THORACIC PRESSURE

**Intra-thoracic Pressure.**—The thoracic cavity, when opened, is far larger than its contents, for the lungs, owing to their elasticity, collapse as soon as the intra-pulmonary and pleural pressures become equal. The intra-pleural pressure is less than the atmospheric pressure by that amount of the atmospheric pressure which is required to overcome the elasticity of the lungs and distend these organs to the size of the thoracic cavity. The intra-thoracic pressure or elastic traction exerted by the lungs on the thoracic wall varies as follows :—

Normal inspiration . . . .	about —	10 mm. Hg.
„ expiration . . . .	„ —	7 „
Deep inspiration . . . .	„ —	40 „
„ expiration . . . .	„ —	0 „
„ inspiration with air-way closed	„ —	100 „
„ expiration „ „ „	„ +	100 „

The intra-tracheal pressure varies from  $-1$  mm. Hg. in quiet inspiration to  $+1$  mm. Hg. in expiration. During forced breathing with the air-way closed the intra-tracheal pressure is greater than the intra-thoracic pressure by the amount of the elastic traction exerted by the lungs. All the structures, e.g. heart and blood-vessels, are affected by the respiratory variations of pressure.

The trachea of a dead rat or rabbit is exposed, and a ligature tied round it. The skin is divided over the thorax on one side, and the ribs exposed. The intercostal muscles are carefully separated between two ribs. Note that the lung is in contact with the thoracic wall. The ligature round the trachea is now divided; the air escapes, and the lung, owing to its elasticity, will collapse. On opening the pleural cavity the pressure within and without the lungs becomes atmospheric. The elasticity of the distended lung then comes into play and causes its collapse. Place a glass tube in the trachea and perform artificial ventilation of the lungs.

## CHAPTER XX

## VENTILATION OF THE LUNGS

## THE SPIROMETER AND THE STETHOGRAPH

The ventilation of the lungs is determined by a gas-meter through which the subject breathes by means of an anæsthetic mask, provided with inspiratory and expiratory valves. Meters with a very

low resistance are more convenient than the special instrument known as the spirometer (Fig. 130), although the latter is very useful for some experiments.

The subject of the experiment should breathe through the mask and meter for a minute or two before the record is taken, in order that he may become accustomed to the novel conditions. Then the volume of each breath and the number in periods of consecutive minutes should be determined. A table should be made to show the results obtained with each member of the class, for the differences in the rate and depth of breathing in healthy men are considerable; some men breathe slowly and deeply, others take rapid and shallow breaths. The volume of air breathed per minute varies from 9 to 5 litres, the number of breaths from 23 to 10, and the averages for the volume of each breath from 900 to 250 c.c. It is important to remember as a general rule that what is lost in frequency is compensated in depth, so that the volume breathed per minute by a man with a frequency of respiration of 10 may be the same as that of a man whose ordinary rate of breathing is 22 per minute.

The **tidal air** is the volume of air breathed at each respiration, and it varies from 900 to 250 c.c. in different individuals. After breathing out the tidal air the subject should expire as deeply as possible; an additional 1,500 to 2,000 c.c. will be recorded. This is called the **supplemental air**. Now let the subject take as deep an inspiration as possible; it will be about 1,500 to 2,000 c.c. above the tidal air. This quantity is known as the **complemental air**.

The so-called **vital capacity** is the greatest volume of air that can be expired after the deepest possible inspiration; it is composed of tidal air 500 c.c. + complemental air 1,500 c.c. + supplemental air 1,500 c.c. It is about 3,500 c.c., but too much importance should not be attached to it, for it depends largely upon practice and control of the inspiratory and expiratory muscles.

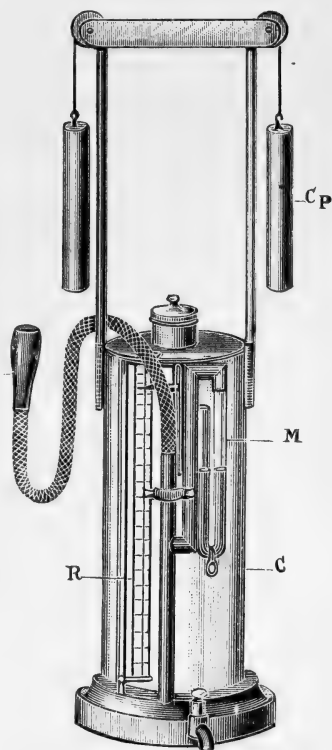


FIG. 130.—Spirometer.

T, mouthpiece; M, manometer;  
Cp, counterpoise; R, scale.

The **Effect of Muscular Exercise upon the Respiration** is very great; within a few minutes, varying according to the severity of the work and the condition of the subject, the volume of air breathed may be trebled, the number of breaths showing a smaller increase. The breathing is deeper, and the mouth is opened to diminish the resistance to the passage of the air in and out of the chest. Discomfort or distress is caused by any resistance, and for this reason it is impossible to determine the true volume unless the resistance of the recording apparatus is low. Place an "anæsthetic" bag between the meter and the tube from the mask in order to reduce the resistance and determine the volume and rate of respiration before and after running down and up a flight of stairs.



FIG. 131.—Stethograph.

A, Metal drum; B, hooks for tapes which pass round neck; C, rubber discs; D, hooks for attaching tapes which are tied round thorax; E, tube leading to the recording tambour.

**The Graphic Record of the Respiratory Movements.**<sup>1</sup>—For this purpose an instrument known as the *stethograph* is used. There are various forms, one of which is shown in Fig. 131. A receiving tambour constructed like a drum is fastened to the chest, and is connected with a recording tambour, the lever of which writes on a smoked drum. The subject of the experiment should not be allowed to see the movements of the lever, for the respiration is easily affected by nervous impressions. Take a graphic record of the respirations and mark the time relations of inspiration and expiration by means of a chronograph giving seconds. Swallow and note that the respiratory movement, inspiratory or expiratory as the case may be, is inhibited.

<sup>1</sup> For other methods see Part I, p. 72.

CHAPTER XXI

CHEMISTRY OF RESPIRATION

**The Composition of Inspired Air, Expired Air and Alveolar Air.**—For the analysis of these different samples of air the best apparatus is that of Haldane. The gas is measured in the graduated gas-burette A, provided with a three-way tap. Surrounding the gas-burette is a water-jacket. The whole is supported by a clamp and retort stand. The gas-burette is connected by pressure tubing to the levelling tube B, which is held by a spring clamp attached to the retort stand. The tubes A and B contain mercury, and by raising or lowering B gas can be expelled from or drawn into A. One of the connections of the three-way tap is used for taking in the sample, the other connects the burette with an absorption apparatus arranged as in the figure.

The bulb E, filled with 20 per cent. caustic potash, absorbs carbon dioxide. The bulb F, filled with alkaline pyrogallic acid solution,<sup>1</sup> absorbs oxygen. The water in G and H protects the pyro solution from the air. F can be emptied and refilled through K when it is necessary. The tap on the absorption pipette places either E or F in connection with the gas-burette.

The pressure in the burette is adjusted by using the potash pipette as a pressure gauge and bringing the potash before every reading of the burette to the mark M. In order to make

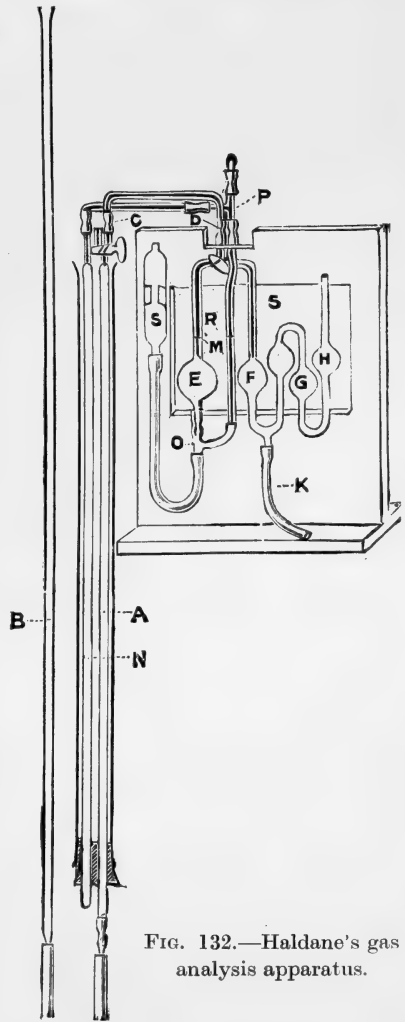


FIG. 132.—Haldane's gas analysis apparatus.

<sup>1</sup> Dissolve 100 grms. of stick caustic potash in 50 c.c. of water. Add 10 grms. of pyrogallic acid to this solution.

the reading of the burette independent of changes in temperature and barometric pressure during analysis a control tube N connected with the potash solution by means of a T-tube is employed.

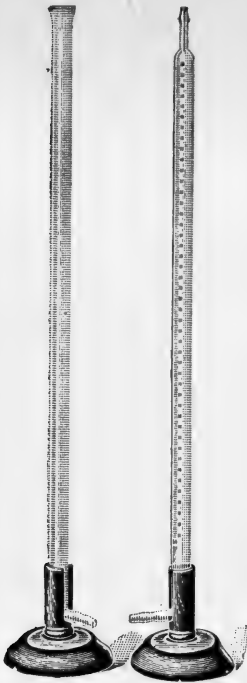


FIG. 133. — Hempel's burette for collecting a sample of expired air.

The tap at P makes it possible to render the pressure in N equal to that of the atmosphere. At the beginning of the experiment the potash is adjusted to the mark R by altering S, P being open. P is then closed, and not opened again till the analyses are complete. The barometer and the temperature of the water-jacket are read. Each time a reading of the burette is made the potash is brought to the mark R by altering S, and to the mark M by means of the levelling tube B. As the control tube and the gas-burette are kept moist, variations in the tension of aqueous vapour in the burette are also corrected by the control tube.

A sample of expired air is obtained with the burette (Fig. 133), or may be collected in a gas-sampler (Fig. 134).

In order to test the apparatus the student should make several analyses of the air of the room.

Special care should be taken to avoid drawing the absorbent fluids into the taps ; if such an accident should occur the taps and tubes will require a thorough cleaning.

**Atmospheric Air**, measured dry at standard temperature and pressure, 0° and 760 mm., has the following composition :—

Oxygen	.	.	.	.	20.94	volumes per cent.
Carbon dioxide	.	.	.	.	0.03	„ „
Nitrogen	.	.	.	.	78.09	„ „
Argon	.	.	.	.	0.94	„ „

There are also traces of helium, krypton, neon, xenon, and hydrogen. The nitrogen and argon appear to be inert as far as the higher animals are concerned, and in ordinary analyses are given together as nitrogen.

**The Expired Air** varies in composition according to the rate and depth of respiration ; this is shown by the following analyses made by Speck :—



Type of breathing.	Volume of air expired per minute c.c.	Percentage of oxygen.	Percentage of carbon dioxide.
Normal . . . . .	7,527	16.29	4.21
Very shallow . . . . .	5,833	15.50	4.63
Very deep . . . . .	17,647	18.29	3.17

Stated in whole numbers the composition may be given as follows :—

	Volumes per cent.		
	Oxygen.	Carbon dioxide.	Nitrogen.
Inspired air . . . . .	21	(0.03)	79
Expired air . . . . .	16	4	80

There are other differences between inspired and expired air. Under ordinary conditions expired air is warmed nearly to the temperature of the body and is saturated with water vapour ; it has about 6 per cent. of moisture, whereas ordinary atmospheric air has about 1 per cent.

The expired air is a mixture of air from the so-called " dead space " of the respiratory tract and of air from the alveoli of the lungs, where the exchange of gases between the blood and the air takes place. The " dead space " extends from the nose to the alveoli and has a capacity of about 150 c.c. in an adult man. In an ordinary expiration the first portion of air to leave the nose or mouth is from this " dead space," then mixed air, and finally air from the alveoli.

**The Alveolar Air.**—The composition of the alveolar air is determined, according to the method introduced by Haldane and Priestley, by an analysis of the last portion of the air expired in an ordinary expiration. The experiment may be performed in the following way. An anæsthetic mask is connected by a T-piece to a piece of tubing 80 cm. long and 1.8 cm. internal diameter ; to the free end of the T-piece is connected (Fig. 134) a gas-sampler with a capacity of 50 cubic centimetres. The subject of the experiment fits the mask to his face and makes an ordinary expiration ; as soon as the expiration ceases, the tap of the gas-sampler, the air of which has previously been removed by a vacuum-pump or gas-pump, is opened and a sample of the last portion of the expired air is collected before the mask is removed from the face. By placing an " anæsthetic " rubber bag at the free end of the tube it is easy to determine whether the subject has made an adequate expiration. The analysis of the air is performed in the manner already described. The percentage composition is about 5.5 carbon dioxide, 14.5 oxygen and 80 nitrogen.

It is an advantage to determine the volume of each expiration by a spirometer attached to the end of the tubing, and it is important that the subject of the experiment should by a little practice with the apparatus learn to breathe naturally, otherwise a fair sample will not be obtained.

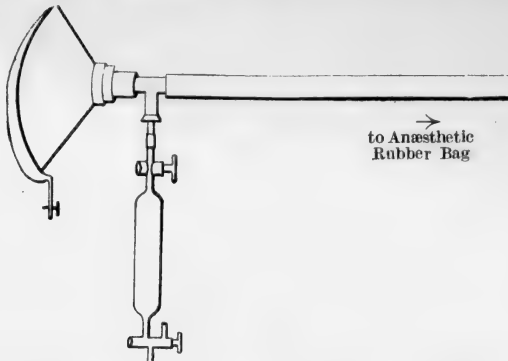


FIG. 134.—Apparatus for collection of a sample of alveolar air.

The partial pressure, or, as it is often called, the tension of the component gases is:—

*Dry atmospheric air:*

Oxygen approximately  $\frac{21}{100} \times 760 = 159.6$  mm. of mercury or 21 per cent. of an atmosphere.

Nitrogen approximately  $\frac{79}{100} \times 760 = 600.4$  mm. of mercury or 79 per cent. of an atmosphere.

Carbon dioxide approximately  $\frac{0.03}{100} \times 760 = 0.228$  mm. of mercury or 0.03 per cent. of an atmosphere.

The tensions of the gases of the alveolar air are calculated in a similar way, but the tension of aqueous vapour must be deducted from the pressure of the atmosphere.

## CHAPTER XXII

### DETERMINATION OF THE RESPIRATORY EXCHANGE IN MAN <sup>1</sup>

An estimation of the intake of oxygen and output of carbon dioxide can be made by analyses of samples of the air expired into a Douglas bag. Collect a sample of expired air and analyse it; then determine by means of a meter the volume of air breathed in

<sup>1</sup> For further details see "The Methods for the Estimation of General Metabolism," Part II.

a minute. The methods involved have been described in previous chapters. From the data obtained a calculation can be made as follows :—

The man breathes 7 litres per minute, and the composition of the expired air was 16 per cent. oxygen and 4 per cent. carbon dioxide ; he had, therefore, absorbed  $21 - 16 = 5 \times \frac{7000}{100} = 350$  c.c. of oxygen and discharged  $4 \times \frac{7000}{100} = 280$  c.c. of carbon dioxide. His respiratory quotient, the ratio of the volume of carbon dioxide discharged to the volume of oxygen absorbed is  $\frac{\text{CO}_2}{\text{O}_2} = \frac{280}{350} = \frac{4}{5} = 0.8$ .

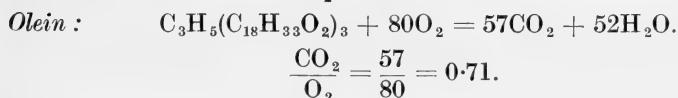
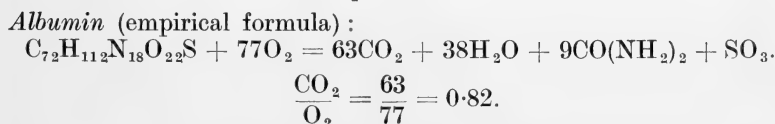
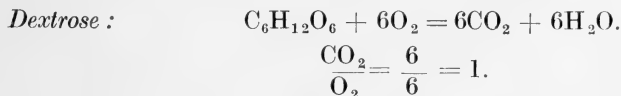
There is a decrease of about  $\frac{1}{30}$  in the volume of the expired air as compared with the inspired air, when both are measured at 0° and 760 mm. ; the deficit is due to the absorption of a small quantity of oxygen which does not reappear in combination with carbon as carbon dioxide, but passes out of the body in other products of oxidation. The increased proportion of nitrogen in the expired air must be taken into account when the respiratory quotient is calculated from volumetric analysis ; thus for every 100 c.c. of expired air the lightly larger volume of inspired air contained the following volume of oxygen :

$$\text{O}_2 = \frac{20.94 \times \text{Nitrogen of expired air}}{79.07}$$

The respiratory quotient, therefore, in a case in which the percentages of nitrogen, oxygen and carbon dioxide are 80, 16 and 4, would be correctly calculated as follows :—

$$\begin{aligned} \text{Oxygen of inspired air} &= \frac{20.94 \times 80}{79.07} = 21.18 \text{ c.c.} \\ \text{Oxygen absorbed} &= 21.18 - 16 = 5.18 \text{ c.c.} \\ \text{Respiratory quotient} &= \frac{\text{CO}_2}{\text{O}_2} = \frac{4}{5.18} = 0.77. \end{aligned}$$

The respiratory quotient varies according to the nature of the food which undergoes oxidation in the body ; thus, for carbohydrates it is 1, for protein 0.8, and for fat 0.7. The following formulæ represent the oxidation of these different substances :—



The respiratory quotient in a living organism is the resultant of various chemical changes and may be complicated by the forma-

tion of fat from carbohydrate and the reverse change, carbohydrate from fat.

The effect of muscular exercise upon the respiratory exchange is most marked; hard work may increase it five to ten times.

For certain researches upon the respiratory exchange of man a respiration chamber is required. Few laboratories possess such an expensive apparatus, but the principles can be studied in the simple form of respiration apparatus for mice.

## CHAPTER XXIII

### RESPIRATION APPARATUS

#### The Haldane-Pembrey Respiration Apparatus for the Mouse.—

The apparatus is constructed as in Fig. 135. Each double absorption tube is fitted with a wire loop, so that the glass need not be touched with the hand. The animal chamber—a light beaker—is provided with a thermometer and is also fitted with a wire loop. The moisture given off by the animal is absorbed by pumice saturated with sulphuric acid in the tubes AB. The carbon dioxide is removed by soda lime in the tube C, and the water given off by the soda lime is caught by the sulphuric acid tube D.

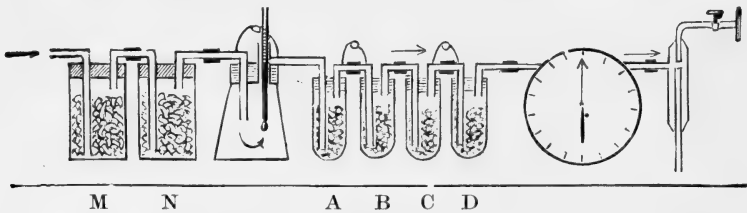


FIG. 135.—The Haldane-Pembrey respiration apparatus for the mouse.

The animal is weighed in the beaker (with the tubes closed) before and after the experiment. A dummy beaker is placed in the opposite scale pan. The tubes AB and CD are also weighed against a dummy pair of tubes. During the weighings the exit and entrance tubes are left unstoppered. By these means errors due to condensation of moisture and changes of barometric pressure or temperature are avoided, and the weighings can be carried out to less than a milligramme.

The air entering the chamber is freed from carbon dioxide and water by soda lime in M and sulphuric acid pumice in N. The amounts of water and carbon dioxide given off in fifteen minutes are determined by the increase in weight of AB and CD respectively. The amount of oxygen absorbed is found by subtracting the loss in weight of the animal weighed in the chamber from the total loss of carbon dioxide and water, for the animal absorbs

during the experiment oxygen and loses water and carbon dioxide.

The ratio  $\frac{\text{CO}_2 \text{ grms.}}{\text{O}_2 \text{ grms.}} \times \frac{32}{44} = \frac{\text{CO}_2 \text{ by volume}}{\text{O}_2 \text{ by volume}} = \text{respiratory quotient.}$

The effect of external temperature upon the respiratory exchange may be studied with this apparatus.

**EXAMPLE.**—The beaker containing a full-grown mouse was placed in a water-bath at 9.5° C.; the mouse gave off from 250–315 decimgrms. of carbon dioxide per ten minutes, and was *active*.

When the temperature of the bath was 30° C. the mouse gave off 103–116 decimgrms. carbon dioxide per ten minutes, and was *quiet*. The rectal temperature of the animal scarcely varied during the experiment. Mammals born in a helpless condition, naked and blind, such as rats and rabbits, behave like cold-blooded animals, and are unable to compensate for low external temperature by increased metabolism; the output of carbon dioxide sinks as their body temperature falls.

## CHAPTER XXIV

### THE CHEMISTRY OF RESPIRATION. THE GASES OF THE BLOOD

In a former chapter experiments were given to prove that the air which is taken into the lungs loses a portion of its oxygen and gains carbon dioxide; these changes correspond to differences in the gaseous contents of the blood; the venous blood loses carbon dioxide and gains oxygen in passing through the lungs, and thus becomes arterial. Analysis shows that blood contains about 60 volumes per cent. of gas, thus 100 volumes of arterial blood will yield 20 volumes of oxygen, 40 of carbon dioxide, and about 1 of nitrogen; 100 volumes of venous blood will yield 12 volumes of oxygen, 48 of carbon dioxide, and 1 of nitrogen.

**Extraction and Analysis of the Gases of the Blood.**—There are many forms of pump for the extraction of the gases of the blood; the general principle is the exposure of the blood to a barometric vacuum. It will be sufficient for the student to work with the simple form of pump introduced by Leonard Hill. For other methods see *The Respiratory Function of the Blood*, by J. Barcroft.

The pump consists of a mercury reservoir A, which is connected with a second reservoir B by means of pressure tubing. The connection is surrounded by a mercury cup. The upper end of B is closed by a three-way tap fitted with mercury cups. By means of this tap B can be put in connection with either the tube E leading to the blood-receiver F, or with the tube C leading to the eudiometer H. The blood-receiver F is constructed of three bulbs, so as to prevent the blood frothing over into B during the extraction of the

gases. On the lower end of F is a three-way tap. To the upper end of F is fixed a piece of thick small-bored pressure tubing provided with a clip.

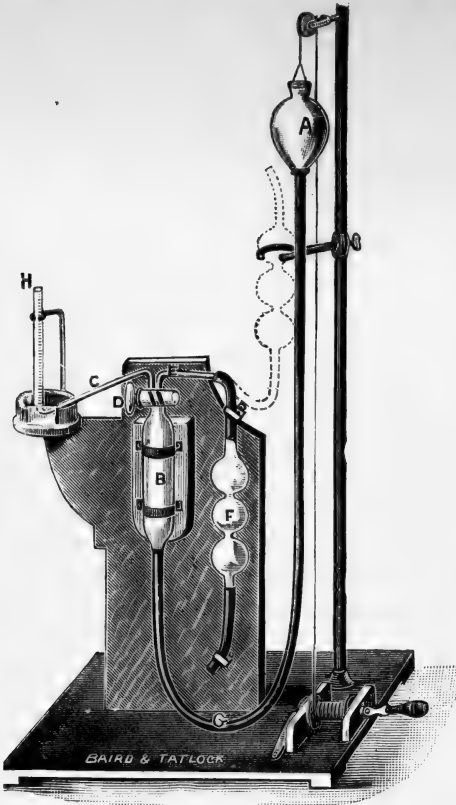


FIG. 136.—Leonard Hill's blood-gas pump.

The mercury used to fill the pump must be cleaned and the pump evacuated before use. In using the pump the manipulations are as follow : F is placed in the position indicated by the dotted line. A is raised and B is put in connection with F, and F is filled with mercury. The clip on the rubber tube at the upper end of F is then closed, and A lowered until F is exhausted, except for 2 or 3 c.c. of mercury which are purposely left within. The screw-clip on the lower end of F is next closed, and F is then detached from the pump and weighed. A sample of blood is, with due precautions, now withdrawn by opening the clip into connection with F. It is now detached, and the blood is defibrinated by shaking it with the mercury left within F for the purpose. F is then again weighed, and the weight of the sample obtained. F is next affixed to the tube E, and E is exhausted. Finally the screw clip between E and F is opened, and the gases are withdrawn and collected in the eudiometer. To facilitate the escape of the gases F is placed in warm water and shaken. If the blood froths too violently the frothing can be allayed by pouring some warm water on the tube E. The

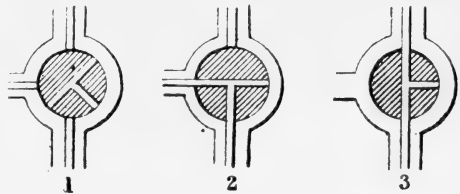


FIG. 137.—The three-way tap of the mercury pump.

tap is so manipulated that the gases only, and not the water which condenses in B, are driven over into the eudiometer. The water is returned back into F. Several exhaustions are needed to extract the gases. The eudiometer tube is filled with mercury and surrounded with a water-jacket to keep the temperature constant. The eudiometer is transferred to a vessel of mercury and the volume of gas read, the level of mercury inside and outside the eudiometer being the same. The temperature of the water in the jacket of the eudiometer is also read and the barometric pressure. Potash solution 20 per cent. is then introduced into the eudiometer by means of a pipette provided with a bent end. The carbon dioxide is thus absorbed and the difference in volume read. Pyrogallic acid is then introduced and the oxygen absorbed. The remainder is nitrogen. The temperature of the water-jacket is kept constant by adding cold water during the estimation. To correct the volume of gas to 0° and 760 mm. the following formula is employed :—

$$V = \frac{V'}{1 + t \cdot 0.00367} \cdot \frac{H - f}{760}$$

where H = the observed pressure, *f* the tension of aqueous vapour at the observed temperature *t*. The value of  $1 + t \cdot 0.00367$  and of *f* are obtained from tables (cf. Sutton's *Volumetric Analysis*).

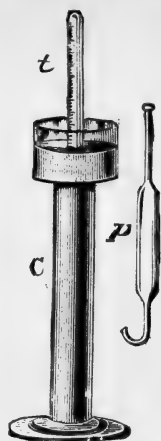


FIG. 138.—C, mercury vessel; t, eudiometer; P, pipette.

## CHAPTER XXV

### THE OXYGEN CAPACITY OF BLOOD

**The Ferricyanide Method of Determining the Oxygen Capacity of Blood.**—Haldane has introduced a simple method of determining the oxygen in combination with the hæmoglobin of the blood. It depends upon the fact that the combined oxygen is liberated rapidly and completely on the addition of a solution of potassium ferricyanide to laked blood. The gas can be easily collected and measured with apparatus similar to that of Dupré for the determination of urea in urine.

The apparatus used by Haldane is shown in Fig. 139.

The process is conducted in the following way :—20 c.c. of oxalated or defibrinated blood, thoroughly saturated with air, are measured from a pipette into the bottle A. To this are added 30 c.c. of a weak solution of ammonia made from ordinary strong ammonia solution, sp. gr. 0.88, by diluting with distilled water to  $\frac{1}{50}$ th.

The ammonia prevents the evolution of carbon dioxide and the distilled water laves the corpuscles. The mixture is thoroughly shaken to complete the laking. Into the tube B are placed 4 c.c. of a freshly saturated solution of potassium ferricyanide. The rubber cork is inserted into the bottle A and the water in the burette is brought to a level close to the top by opening the tap and raising the levelling tube. The tap is closed and the reading of the burette taken. The water gauge attached to the temperature and pressure control tube is adjusted by sliding the rubber tubing backwards or forwards on the glass tube D.

The bottle A is tilted so that the ferricyanide in B escapes and

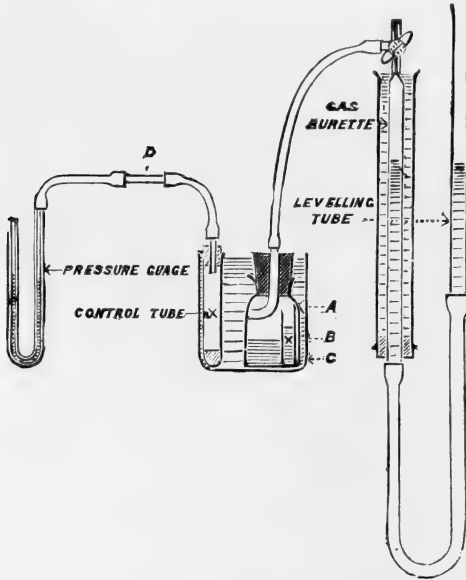


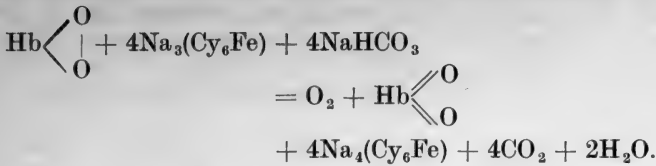
FIG. 139.—Ferricyanide method of estimating the oxygen capacity of blood.

the mixture is shaken until the evolution of gas has ceased. If the pressure gauge indicates an alteration in the temperature of the water this is adjusted by the addition of cold or warm water to the bath. After allowing the temperature to become constant and levelling the water in the burette and levelling tube, the amount of gas is read. The temperature of the water surrounding the burette and the height of the barometer are taken and the gas is reduced to its volume at  $0^{\circ}$  and 760 mm.

The chemistry of the process appears to be as follows:—The ferricyanide is reduced to ferrocyanide, for if ferricyanide be added to laked blood it will be found that the solution gives with ferric chloride the blue colour which indicates the presence of ferrocyanide.



Oxygen is rendered available for the formation of methæmoglobin after the oxygen of the oxyhæmoglobin has been liberated.



In this case  $\text{Hb} \begin{array}{c} \diagup \text{O} \\ | \\ \diagdown \text{O} \end{array}$  represents oxyhæmoglobin, and  $\text{Hb} \begin{array}{c} \diagup \text{O} \\ | \\ \diagdown \text{O} \end{array}$

methæmoglobin, for it is held that the oxygen atoms are united together in oxyhæmoglobin but not in methæmoglobin. According to some authors methæmoglobin contains half as much oxygen as is present in oxyhæmoglobin and should be represented by the formula  $\text{Hb} = \text{O}$ .

CHAPTER XXVI

BLOOD. THE HÆMOGLOBINOMETER, THE HÆMA-CYTOMETER AND HÆMATOCRITE

**Gowers-Haldane Hæmoglobinometer.**—The maximal error of this admirable instrument is not more than 0·8 per cent. The

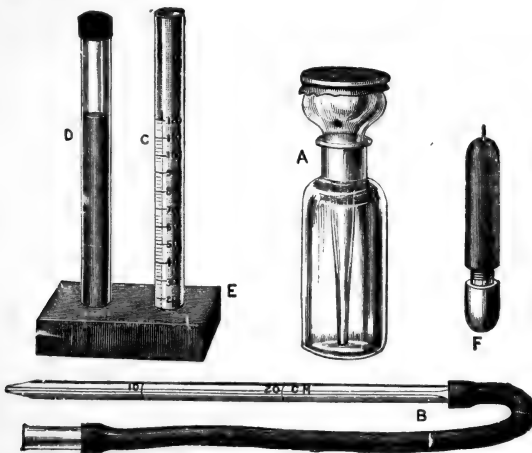


FIG. 140.—Hæmoglobinometer.

standard solution in tube D is a 1 per cent. solution of ox blood saturated with coal gas.<sup>1</sup> The oxygen capacity of the ox blood from which the standard was prepared was 18·5 per cent. This

<sup>1</sup> Coal gas contains carbon monoxide as an impurity.

was determined by displacing the oxygen from laked ox blood with ferricyanide of potassium, and measuring the amount of gas. The percentage of hæmoglobin corresponding to 18·5 per cent. is about 13·8 per cent. The normal human blood when saturated with CO and diluted with water to the mark 100 in tube C corresponds in tint to the standard, and has therefore an oxygen capacity of 18·5 per cent.

Add distilled water to tube C up to the mark 20. Take exactly 20 c.mm. of blood in the pipette, and blow it into C. Pass a narrow glass tube connected with a gas burner into the free part of tube C. Turn the gas on and push the glass tube down near to the blood. The gas tube is then withdrawn, and tube C quickly closed with the finger to prevent the gas escaping. The tube is then inclined up and down about a dozen times, so that the hæmoglobin becomes saturated with CO.

Distilled water is then added drop by drop from the dropping pipette A, until the tint appears equal to the standard. After half a minute read the percentage, and then add another drop or drops till the tints appear just unequal. Read the percentage again, and take the mean of the two readings as correct. In comparing the tints hold the tubes against the skylight, and frequently change the tubes from side to side.

**The Number of Corpuscles in the Blood.**—The Thoma-Zeiss **Hæmacytometer** consists of a counting chamber and an accurately calibrated pipette.

The finger behind the nail is cleaned with alcohol and ether, and a drop of blood is drawn by the stab of a lancet-shaped needle. The finger should not be constricted by a ligature during this operation. The point of the pipette is placed in the drop, and the blood is aspirated as far as the mark 1. The traces of blood on the point of the pipette are then removed, and the pipette is dipped into Hayem's fluid.<sup>1</sup>

This fluid is sucked up until the diluted blood reaches the mark 101. The tip of the mouth-piece is then closed by the finger, and the pipette shaken. The glass bead in E mixes the blood and Hayem's fluid. The bulb contains 1 part blood and 99 Hayem's fluid.

Now blow gently into the mouth-piece, reject the first few drops, and then place a drop upon the centre of the counting chamber. The cover-slip is then placed in position, and the counting chamber is placed on the stage of the microscope, and left at rest for a few minutes. When the corpuscles have subsided, count the number in 10 squares, and take the average. Count those corpuscles which happen to lie on the lines on two sides of each square only. Each square covers an area of  $\frac{1}{400}$  sq. mm., and has a volume of  $\frac{1}{4000}$  c.mm., therefore 1 c.mm. contains 4000 times the average number found in a square. The dilution of the blood was 1·100. Thus

<sup>1</sup> Sodium chloride, g. 2; sodium sulphate, g. 10; corrosive sublimate, g. 1; water, g. 400.

the number in a square  $\times 4000 \times 100 =$  number of corpuscles in 1 c.mm. of blood.

In counting the white corpuscles it is best to dilute the blood with 1 per cent. acetic acid. This destroys the red corpuscles and brings the white clearly into view. By comparing the number of the red corpuscles in a square with the percentage of the hæmoglobin, the worth of the corpuscle in hæmoglobin is obtained.

$$\frac{\% \text{ of Hb}}{\text{No. in sq.}} = \text{“worth” of corpuscles.}$$

The average number of red corpuscles is 5,000,000 per 1 c.mm. ; of white, 10,000 per 1 c.mm.<sup>1</sup>

**Specific Gravity of the Blood.**—A number of test tubes are taken and filled with mixtures of glycerine and water, which vary in specific gravity from 1030 to 1075. A pipette is taken with the point bent at a right angle. The skin is pricked behind the finger

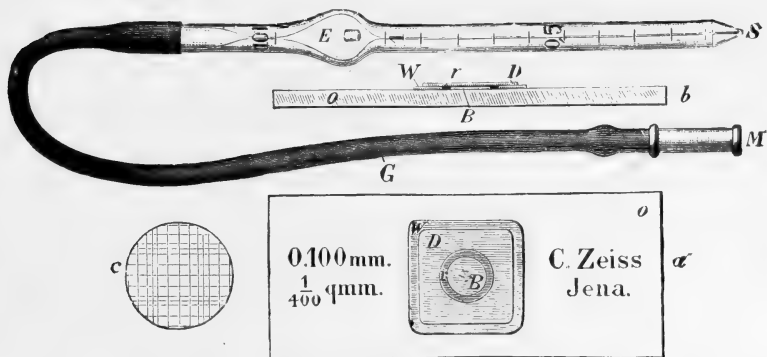


FIG. 141.—The Thoma-Zeiss hæmacytometer.

nail, and a drop of blood is drawn into the pipette. The blood is blown in small droplets into the middle of the solution in several of the test tubes until the solution is found in which the blood neither sinks nor rises. The specific gravity of this solution is determined with the hydrometer. The behaviour of the droplet must be noted at the moment when it enters the solution. The blood quickly alters owing to osmotic change. The specific gravity of the blood is about 1060, of the plasma 1026–29. The specific gravity of fragments of muscle or other tissues may be determined in the same way. The method is thus employed to determine the amount of tissue-lymph in the organs.

In place of the mixture of glycerine and water a mixture of chloroform and benzol may be used. By the addition of either

<sup>1</sup> After using, clean the pipettes of these instruments. Suck water, alcohol, and ether up them in turn, and let the liquids run out. Never blow down the pipettes.

fluid a mixture can be obtained in which the drop of blood remains suspended. The specific gravity of the mixture at this stage is determined by a small hydrometer.

**Hæmatocrite.**—The relative volumes of corpuscles and plasma may be determined by centrifugalising a small quantity of blood in a graduated glass tube of narrow bore (hæmatocrite). The rotation must be at a high speed (10,000 revolutions per minute) in order to bring about the separation of the corpuscles from the plasma before the blood clots. The usual proportions are plasma two-thirds, corpuscles one-third.

## CHAPTER XXVII

### THE INFLUENCE OF CARBON MONOXIDE

Carbon monoxide is a poisonous gas in virtue of its great affinity for hæmoglobin; oxygen is displaced and carboxyhæmoglobin is formed. Unconsciousness, convulsions, and death are produced by the lack of oxygen which arises when a large portion of the hæmoglobin is combined with carbon monoxide and thus deprived of its power of carrying oxygen.

Carbon monoxide is present as an impurity in coal-gas, and in water-gas, which is often used in the adulteration of coal-gas, the percentage is a very high one. It is due to this gas that death so often results from coal-gas poisoning. In the air of mines after an explosion there is present a large quantity of carbon monoxide, due to the incomplete combustion of coal-dust; miners overtaken by such a disaster generally die from poisoning by this gas.

**DEMONSTRATION.**—A white rat or mouse is selected for the experiment, for it is easier in such animals to see in the snout and feet the change of colour due to the formation of the carbonyhæmoglobin. The animal is placed under a glass bell jar and coal-gas is admitted; it becomes restless, unconscious, convulsed, and dies within a few seconds. This is one of the quickest methods of killing an animal, and has the advantage that it rapidly produces unconsciousness.

If the animal be removed to free air at the beginning of the stage of unconsciousness it may recover. The carboxyhæmoglobin is gradually dissociated and oxyhæmoglobin is formed in its place. In rabbits this occurs very rapidly; the animal quickly recovers, passing through a stage of incoordination.

Haldane has shown that the best indicator of the presence of poisonous doses of carbon monoxide is a small warm-blooded animal, such as a mouse or bird, which is affected, owing to its rapid respiratory exchange, much sooner than a man. This method has been employed with success by rescue parties entering a coal mine after an explosion.

The colour of the snout and feet of the white mouse or rat killed

by carbon monoxide is pink or cherry red. The blood in the viscera has a similar colour and the contrast between the appearance of an animal killed by ordinary asphyxia produced by a blow on the head and one killed by lack of oxygen due to carbon monoxide is very striking.

Perform this simple and practical test for carbon monoxide. Kill two animals, one by a blow on the head, the other by coal-gas. Cut open their bodies and compare the colours of the viscera. Place a drop of blood from each animal in separate test tubes, dilute with distilled water and examine in good daylight. The blood containing carboxyhæmoglobin can be distinguished easily by its cherry red colour; it is more pink and less yellow than the ordinary diluted blood. This test can be confirmed by the examination of the two samples of blood with the spectroscope.

The treatment of cases of carbon monoxide or coal-gas poisoning is to give oxygen to increase the dissociation of carboxyhæmoglobin and to keep the patient warm in order that his metabolism and the excitability of his nervous system may be raised.

## CHAPTER XXVIII

### THE REGULATION OF RESPIRATION

The ventilation of the lungs is regulated by a nervous centre in the medulla oblongata. This can be proved by a series of experiments, in which different portions of the central nervous system are destroyed.

**DEMONSTRATION.**—The medulla of an anæsthetised mammal is destroyed in the region of the *calamus scriptorius*; respiration ceases immediately and the animal dies of asphyxia.

By experiments upon other animals it can be proved that destruction of no other part of the central nervous system will produce this sudden cessation of all respiratory movement. If the spinal cord be divided close to the medulla the chief respiratory muscles will be paralysed, but the movements of the nares will show that the centre is not destroyed.

The respiratory centre is influenced in two ways: (i) by the composition of the blood which supplies it, and (ii) by nervous impulses which affect its excitability. Experiments upon these points can be performed by the student upon himself; he can alter the composition of the air in his lungs and thus affect the gaseous composition of his blood.

**Influence of Breathing Air Containing Carbon Dioxide.**—The subject of the experiment breathes air through a mask and valves and the ventilation of the lungs is determined by a meter. Then, unknown to the subject, the air to be breathed is taken from a gas bag containing air with 3 or 4 per cent. of carbon dioxide. The breathing is increased. Carbon dioxide stimulates the respiratory

centre. In order to check any effects of change in resistance or of suggestion the gas bag should, unknown to the subject, be filled with pure air and the experiment repeated. Air containing 8 or 9 per cent. of carbon dioxide will produce intolerable discomfort or distress.

**Influence of Breathing Different Percentages of Oxygen.**—After breathing air for some time, the subject breathes pure oxygen from a bag: the rate and volume breathed generally show no change, if precautions have been taken to avoid the effects of suggestion. If the oxygen be moistened with water most men cannot distinguish it from air taken from a similar bag.

Air containing about 15 per cent. of oxygen can be collected free from carbon dioxide by breathing slowly through a flask or tin of soda lime into a gas bag. Experiments with this gas will show no change in the rate or volume of the air breathed. A fall of 5 or 6 per cent. in the amount of oxygen in the air is not detected. When the oxygen is only 10 per cent. effects are produced; these will be studied in later experiments.

**Influence of Holding the Breath.**—Hold the breath to the “breaking point” and then collect a sample of alveolar air. The carbon dioxide will rise to 7 or 8 per cent.; the oxygen will fall to about 10 per cent.

Repeat the experiment after breathing oxygen for two or three minutes. The “breaking point” will not occur so soon, but the rise in the carbon dioxide will be the determining factor, for the oxygen in the alveolar air may be above 20 per cent. at the end of the experiment. The carbon dioxide may rise to 10 per cent.

**Influence of Forced Breathing.**—Take a series of rapid and deep breaths for about half a minute, recording the movements by the stethograph.<sup>1</sup> Stop breathing when a sensation of giddiness is experienced. There will be no inclination to breathe for about a minute. The condition is one of *apnœa*, due to the washing out of carbon dioxide from the lungs and blood. The composition of the alveolar air will indicate the changes which occurred, as shown by the following example. The subject breathed rapidly and deeply, seventeen times in eighteen seconds. A sample of alveolar air from the last expiration yielded on analysis 2.50 vols. per cent. of carbon dioxide and 19.23 of oxygen. Apnœa followed. The sample of the first expiration, when a desire to breathe was felt, had the following composition: carbon dioxide 5.59 vols. per cent., oxygen 12.59 per cent.

The experiment should then be repeated, with this difference: oxygen instead of air should be breathed. The period of apnœa will be much longer, for the subject of the experiment will have more oxygen in his lungs and more in his venous blood.

Forced breathing interferes with the circulation and often produces giddiness. An examination of the pulse will show that the

<sup>1</sup> See p. 134.

systolic pressure is diminished by each inspiration. If oxygen is taken in during forced breathing there is less discomfort ; the brain receives more oxygen even if its circulation of blood is disturbed.

**Influence of Muscular Exercise.**—The subject of the experiment should take vigorous muscular exercise sufficient to produce hyperpnœa, but not long enough for the production of “second wind.” A sample of alveolar air taken immediately after the exercise will show in many cases a considerable rise in the percentage of carbon dioxide and a small fall in that of oxygen. If the exercise be continued until “second wind” has been established, the alveolar air will show less carbon dioxide and more oxygen. This accommodation varies in different subjects, but the following example may be given.

PERCENTAGE COMPOSITION OF ALVEOLAR AIR.

Carbon dioxide. Vols.	Oxygen. Vols.	$\frac{\text{CO}_2}{\text{O}_2}$	
5.27	14.32	0.79	At rest.
7.36	14.03	1.06	After running $\frac{1}{2}$ mile.
5.91	14.62	0.93	After running $\frac{1}{4}$ mile more. “Second wind.” Sweating.

“Second wind” appears to be a complex adjustment of the respiration and circulation to the demands of muscular work.

## CHAPTER XXIX

### CHEYNE-STOKES RESPIRATION

In certain cases of heart disease a well-marked alternation of apnœa and hyperpnœa was observed and described by Cheyne and Stokes. This phenomenon is characterised by a period of waxing and waning respiration followed by a period of apnœa (Fig. 142).

In some healthy men Haldane and Douglas have shown that this type of periodic breathing can be produced in the following way. The subject breathes through a small tin of soda lime provided with wire gauze to prevent the suction of small pieces of soda lime into the mouth and connected at the far end with a piece of tubing about 260 cm. long and of about 2 cm. bore. The subject thus rebreathes his own expired air after it has been deprived of carbon dioxide by the soda lime. The percentage of oxygen necessarily falls, the respiratory centre becomes excited and hyperpnœa begins. Some fresh air from outside the tube will be taken in with each deep breath and the percentage of oxygen will rise. The hyperpnœa, however, has washed out a quantity of carbon dioxide from the blood and air in the lungs, and apnœa results owing to lack of sufficient carbon dioxide to excite the respiratory centre. Thus

this alternation of breathing and apnœa may continue for several minutes or, it may be, hours. Some healthy men exhibit Cheyne-Stokes respiration readily when they perform this experiment; others do not.



FIG. 142.—Cheyne-Stokes respiration. (Pembrey and Allen.)

Apnœa can be abolished by either (i) air containing 3 or 4 per cent. of carbon dioxide, or (ii) pure oxygen, or (iii) air containing a deficiency of oxygen, about 12 per cent. of oxygen.

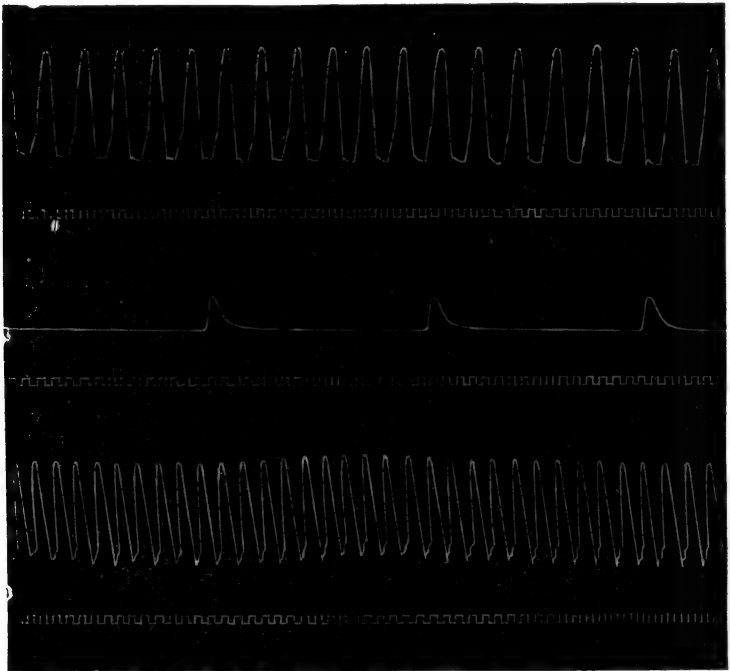


FIG. 143.—Frog's heart.

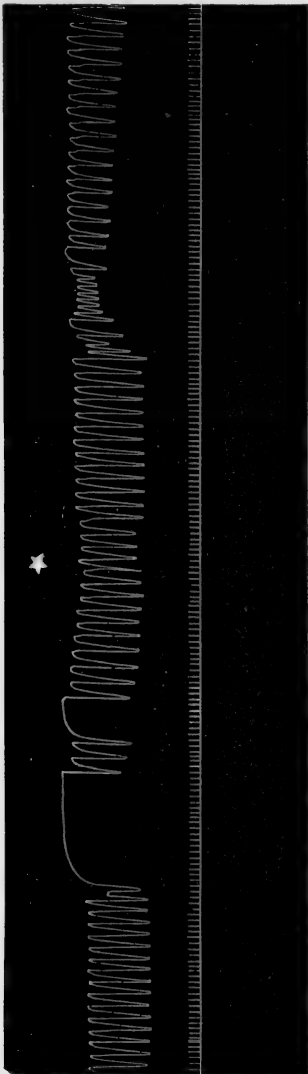
1, Normal; 2, three minutes after one drop of 10 per cent. solution of muscarine; 3, after the application of a weak solution of atropine sulphate. The time is marked in seconds. (Pembrey and Phillips.)



CHAPTER XXX

THE ACTION OF DRUGS UPON THE FROG'S HEART

**Action of Muscarine and Atropine.**—Dissect out the vago-sympathetic nerve and record the effect of excitation of (1) the vago-sympathetic, (2) the crescent. Next with a glass pipette apply



Stimulation before atropine.

Stimulation after atropine.

FIG. 144.—Contraction of the frog's heart.

Effect of excitation of the sino-auricular junction before and after atropine. The atropine was applied at the point started. The time is marked in seconds. The curve should be read from left to right. The downstroke represents contraction. (L.H.)

to the heart a few drops of nitrate of muscarine (10 per cent. solution). The tone, frequency, and amplitude of the heart will decrease until at last the heart becomes arrested in diastole. Mechanical excitation may still excite the heart to give a single contraction.

Now apply some drops of a 0.2–0.5 per cent. solution of atropine sulphate. The heart will begin to beat again, at first feebly, and then with increasing amplitude. Muscarine abolishes the tone, rhythmic power, and conductivity of heart muscle, while atropine

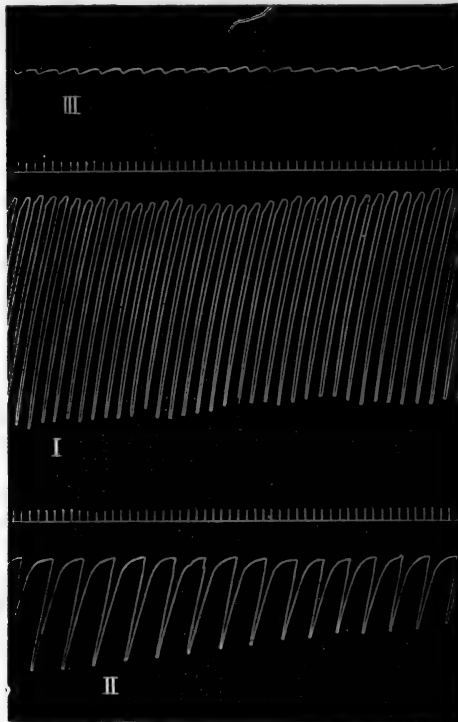


FIG. 145.—Contraction of the frog's heart.

I. Normal heart-beat, II. and III. poisoned by nicotine. The downstroke represents contraction. The time is marked in seconds. (L.H.)

has in each respect the antagonistic action. This experiment succeeds on any ganglion-free strip of the heart of the tortoise. After the application of atropine, excitation, either of the vagus or of the crescent, is ineffectual, for atropine paralyses the post-ganglionic fibres of this nerve. The effect of atropine cannot be antagonised by a further application of muscarine (Fig. 143).

A 1 per cent. solution of pilocarpine acts in the same way as muscarine, and atropine acts as its antagonist.

Muscarine is an alkaloid obtained from the poisonous Fly Agaric

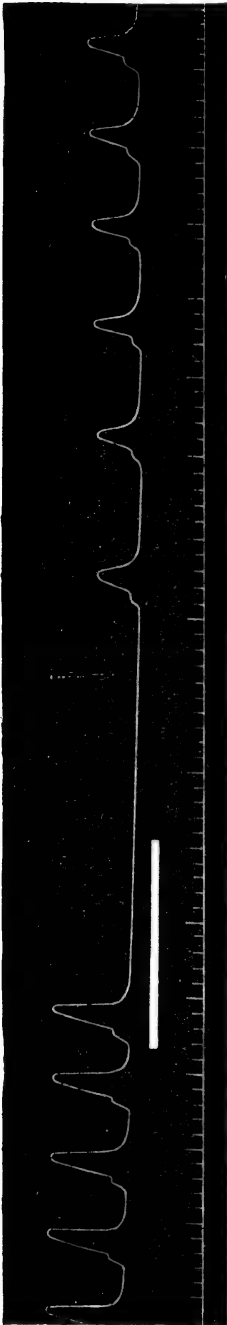


FIG. 146.—Contraction of the frog's heart. Nicotine 1 in 1000 saline. Effect of exciting the sino-auricular junction during the period shown by the white line. Excitation of the vago-sympathetic on the contrary produced no effect. The time is marked in seconds. (Pembrey and Phillips.)

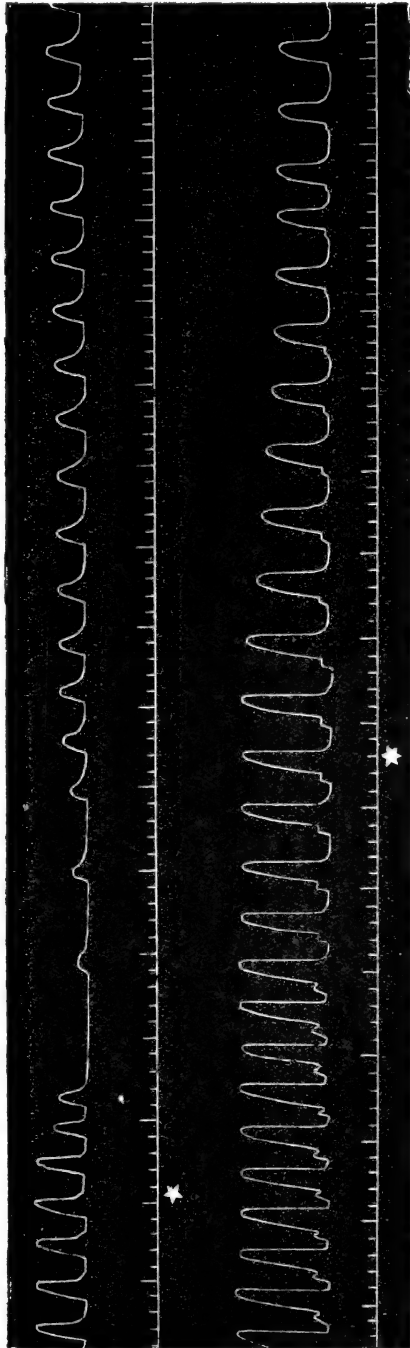


FIG. 147.—Contraction of the frog's heart. Effect of applying a drop of chloroform to the frog's heart at the points starred. The time is marked in seconds. (Pembrey and Phillips.)

—a fungus. It is used as an intoxicant in Siberia. It is excreted, unchanged, in the urine, and it is stated that the urine is drunk when the supply is short, and thus the intoxicant is handed on from one man to another.

Muscarine nitrate,  $C_5H_{15}NO_3$ , is prepared artificially from cholin,  $C_5H_{15}NO_3$ . Cholin is one of the decomposition products of lecithin.

**Action of Nicotine.**—Dissect out the vago-sympathetic and record the beat of the heart by the suspension method. Record the effect of excitation of (1) the vago-sympathetic, (2) the crescent. Now apply to the heart drops of a 0.1 per cent. solution of nicotine. The frequency of the heart is at first lessened and then slightly increased, for the nicotine firstly excites and secondly paralyses the synapses of the vagus fibres with the cardiac ganglia. These ganglia contain the cell stations of the vagus fibres. Stimulation of the vago-sympathetic trunk no longer produces inhibition, but augmentation and acceleration. The cell stations of the sympathetic fibres are in the third sympathetic ganglion.

The vagus fibres are medullated as far as the cardiac ganglia, while the sympathetic fibres are non-medullated after leaving the third sympathetic ganglion (Fig. 148). Stimulation of the crescent still produces inhibition, for weak doses of nicotine do not paralyse the post-ganglionic fibres. Nicotine is similarly employed to determine the cell stations of all the nerve fibres of the autonomic system (Langley). Too large a dose of nicotine paralyses the post-ganglionic fibres, and renders the contraction of the muscle slow. At this stage stimulation of the sinus will cause a series of rapid beats due to the excitation of the cardiac muscle; this acceleration shows as an after-effect a prolonged period of diastole. Nicotine finally arrests the heart-beat by poisoning the muscle.

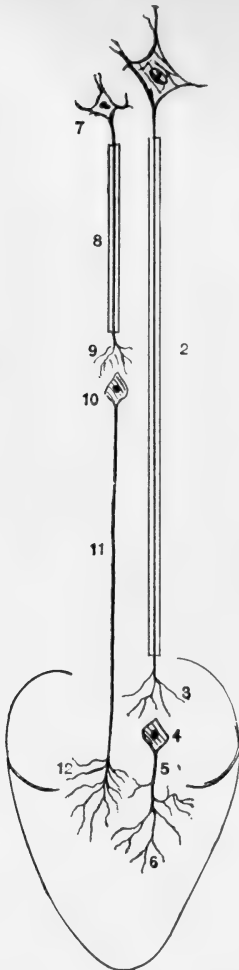


FIG. 148.—Diagram of the nerves of the heart.

1. Nerve-cell of the vagus centre in the medulla oblongata.
2. Medullated inhibitory fibre of the vagus.
3. Its termination around a ganglion-cell (4) in the heart.
4. Post-ganglionic non-medullated fibre and its termination (6) in the muscular tissue of the heart.
5. Ganglion cell giving rise to the medullated acceleratory fibre (8) in the white ramus communicans.
6. The termination of the medullated fibre in the muscular tissue of the heart.
7. The termination of the non-medullated fibre in the muscular tissue of the heart.
8. The medullated portion of the vagus nerve.
9. The non-medullated portion of the vagus nerve.
10. The nerve-cell of the ganglion stellatum.
11. The medullated portion of the sympathetic trunk.
12. The non-medullated portion of the sympathetic trunk.

**Action of Chloroform and Ether.**—Excise two frogs' hearts and place each in a watch glass containing 5 c.c. of Ringer's fluid. To one add one drop of pure chloroform and cover with another watch glass. The heart will become feeble, lose tone, and finally stop beating. It will take considerably more ether to produce the same effect on the other heart. The causation of death from chloroform is cardiac failure. In the mammal the arterial pressure falls, and the vagus centre is rendered hyperexcitable by too concentrated a dose of chloroform. Failure of respiration and syncope result from inhibition and poisoning of the heart.

	By Molecules.	By Weight.	By Volume.
Alcohol . . . . .	1	1	1
Ether . . . . .	8	5	5
Chloroform . . . . .	100	40	75

The relative physiological powers of alcohol, ether and chloroform. (Waller.)

### CHAPTER XXXI

#### PERFUSION OF THE VENTRICLE OF THE FROG'S HEART

The Symes cannula is fastened into the auricles by a thread passed over the dorsal surface of the aortic bulb and tied in the sinu-auricular groove. The side tube of the cannula is connected with the perfusing fluid, which is pumped by the ventricle through the aorta and streams downwards over the surface of the heart. The ventricular beat is recorded by a lever connected to the apex by a light clip.

With this method determine the effects of distilled water, normal tap-water saline or Ringer's fluid (Fig. 149) and of the drugs mentioned in the previous chapter.

### CHAPTER XXXII

#### VASO-MOTOR SYSTEM OF THE FROG

**Innervation of the Blood-vessels.**—Place a frog on the cork board provided for studying the circulation in the web. Observe the rate of the circulation in the web of foot. Destroy the nervous tissues in the cranium and the medulla. The circulation will become more rapid owing to dilatation of the arteries.

Now remove the frog from the board and expose the heart. Suspend the frog in the vertical head-up position. Note that the heart and large vessels are filled with blood. Pass a blanket-pin down the vertebral canal and destroy the spinal cord. The heart and vessels will soon become bloodless owing to the loss of vaso-motor tone. The blood sinks into the dilated abdominal vessels under the influence of gravity.

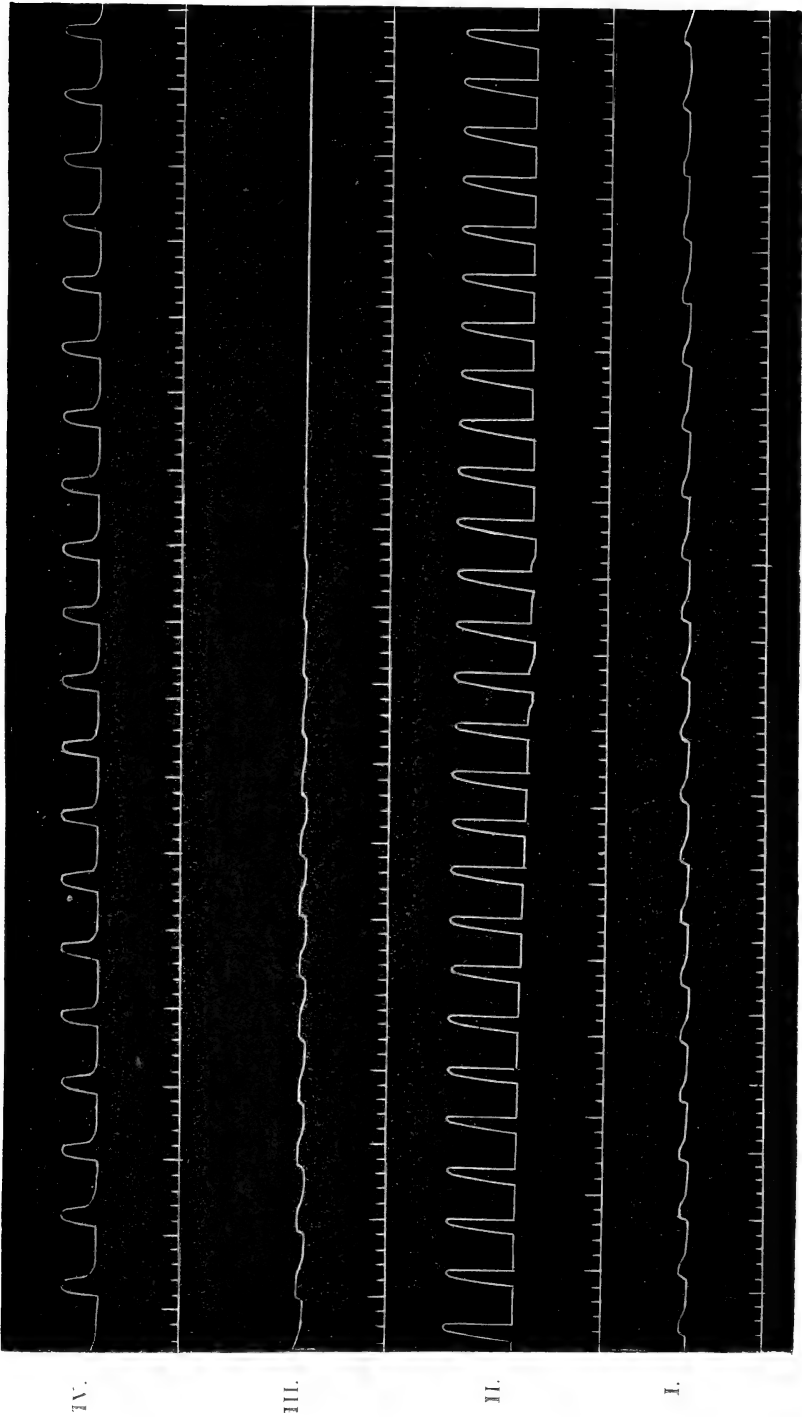


FIG. 149. Contraction of the heart of the frog.

I. Effect of distilled water. Temp., 16.25°. II. Contraction restored by normal tap-water saline. III. and IV. Repetition of the experiment. The time is marked in seconds. (Pembrey and Phillips.)

**Perfusion of Frog's Blood-vessels.**—Destroy the cerebrum and expose the heart. Tie one aorta. Place a ligature under the other, snip it with sharp scissors, and allow the blood to escape. Insert a fine-glass cannula into it pointing away from the heart. Fill the cannula with normal saline by means of a capillary pipette. Connect a rubber tube to a glass funnel and clip the tube. Fill the funnel and tube with Ringer's fluid. Connect the tube with the cannula. No air bubbles must be introduced. Snip the sinus venosus and open the clip. Hang the frog in the vertical position. The fluid circulates, runs out of the sinus, and drops from the toes of the frog into a measure glass. Measure the outflow per minute. Circulate Ringer's fluid plus 1 in 1000 sodium nitrate; the outflow is increased owing to vaso-dilatation. Supra-renal extract produces the opposite effect.

## CHAPTER XXXIII

### CIRCULATION OF THE BLOOD

**Proofs of the Circulation of the Blood.**—The following experiments should be performed upon a decerebrate mammal. A decapitated duck is very useful owing to the great length of neck for dissection and the ease with which cardiographic records of the ventricular contractions may be taken.

The external jugular vein and the carotid artery are exposed and the conditions as regards pressure and pulsation in these vessels are determined carefully by the fingers. A clip is placed on the jugular vein, the central end becomes empty, the peripheral end engorged. The clip is placed next on the carotid artery, the central end becomes distended and pulsates, while the peripheral end shrinks and ceases to pulsate. The clip is removed and two double ligatures are placed in position under each vessel. The vein is pricked; dark blood flows out from the peripheral end steadily and with little force. The vein is ligatured above and below the opening. The artery is pricked; bright blood spurts out forcibly and in jets from the central end. The artery is ligatured above and below the opening.

A cannula is placed in the trachea and connected with the apparatus for artificial respiration. The sternum is divided in the mid-line, and the thorax opened. Observe the inflation and elasticity of the lungs and the contraction of the heart inside the pericardium; feel with the thumb and finger the contraction and relaxation of the ventricles. Slit open the pericardium and observe the heart. Ligatures are passed under the superior and inferior venæ cavæ and tightened; the heart quickly becomes empty. Loosen the ligatures and observe the immediate filling of the right side of the heart. Now a ligature is passed under the aorta and tightened; the left and then the right side of the heart becomes engorged,

Note the effect of loosening the ligature. Now tie a ligature on the pulmonary artery; the right side of the heart becomes engorged and the left empty. Loosen the ligature and observe the effects. The heart is then excised and the pulsations studied.

**The Influence of Gravity on the Circulation of the Eel.**—Pith the brain of an eel. Fasten the animal on to a board. Expose the heart, which may be seen beating beneath the skin, about 2–3 inches below the mouth. Place the animal head down in the vertical position. Notice the pericardium prevents the over-distension of the heart by the weight of the super-incumbent column of blood. Slit open the pericardium and observe the result. The heart becomes greatly congested. This is especially marked in the eel, when reflexly excited to writhe. Turn the animal head uppermost. The heart gradually empties, and becomes at last pale and bloodless. Slowly tilt the board and observe the blood as it runs up the inferior vena cava and fills the heart. Place the animal again in the vertical posture (head up), and observe that the heart fills (*a*) on compressing the abdomen from below upwards, (*b*) on sinking the animal in a bath of water up to the level of the heart. In (*b*) the weight of the water outside tends to balance the weight of the blood within.

The vagus nerve may be found at the side of the neck, and the effect of its excitation noted. Reflex inhibition of the heart is very easily brought about by striking the abdomen or gills, or pinching the tail of the eel.

**Demonstration of Vaso-Motor Nerves.**—A white rabbit is chosen, or one with a white ear; the brain of the animal is pithed and artificial respiration established at once. The cervical sympathetic is exposed in the neck, where it lies behind the carotid artery, and is traced up to the superior cervical sympathetic ganglion. The thread is tied round the nerve, and the latter is cut. Observe that at this moment the blood vessels in the ear dilate and the ear becomes warmer. The palpebral fissure at the same time becomes narrowed. The change will be much more marked had the ear of the rabbit been previously exposed to cold. The cervical sympathetic exercises a tonic action. On exciting the peripheral end of the nerve with the faradic current, the vessels in the ear will be seen to constrict, and this will take place to such a degree that all the smaller vessels will disappear from view. The ear will at the same time become several degrees cooler. Note that the latent time is considerable between the excitation and the effect. Note that the pupil also dilates, the nictitating membrane retracts, and the palpebral tissue is widened. The eyeball at the same time projects forwards. The pupillo-dilator fibres arise from the first three thoracic anterior roots, the vaso-constrictor fibres from the second to the fifth, and even to the seventh, in the rabbit. If the superior cervical sympathetic ganglion be painted with nicotine, excitation of the perganglionic fibres will no longer have any effect



on the ear, while excitation of the post ganglionic fibres will still be effectual. The sympathetic fibres to the head have their cell-stations in this ganglion.

**The Circulation Time of the lesser Circulation.**—The carotid artery is exposed. A piece of thin rubber membrane is placed beneath it. Between the membrane and the artery a piece of white paper is inserted. The artery is illuminated by a strong light.

The external jugular vein is exposed on the other side of the neck, a clip is placed on the vein below and it is tied above, and

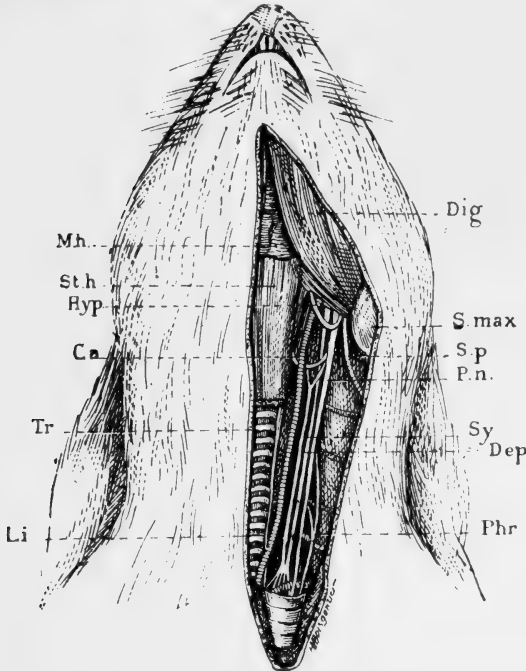


FIG. 150.—Dissection of the vagus (P.n.), the depressor (Dep.), and cervical sympathetic (Sy.) nerves in the rabbit. (Livon.)

into its central end a cannula is inserted. The vein cannula is connected with a glass syringe containing a 0.2 per cent. solution of methylene blue in physiological saline at body temperature. Put a screw clip on the piston so that one-third of the contents shall be ejected. The clip is removed from the vein and at a signal from the assistant who times the experiment the syringe is pressed. The stop-watch is stopped by the assistant the moment the blue appears in the artery. The observation is repeated several times with the same amount of injection.

**Record of Arterial Pressure, Effect of Excitation of the Vagus and**

**Depressor Nerves. Effect of Asphyxia.**—A cannula is placed in the carotid artery and is connected to a mercurial manometer by a piece of pressure tubing, a  $\perp$  piece being interposed. The cannula and tube are filled by means of a pressure bottle or syringe with

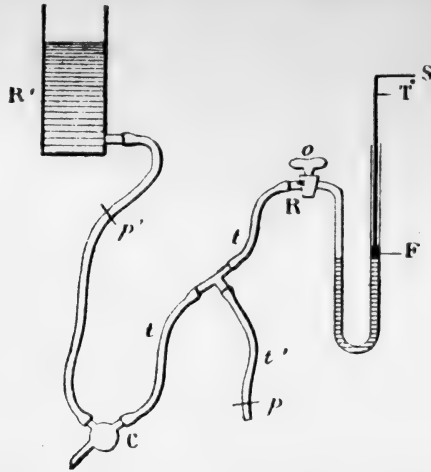


FIG. 151.—Arrangement of cannula, pressure bottle, and mercurial manometer for recording blood pressure.

C, cannula;  $p$ ,  $p'$ , clips; F, float; S, writing style.

sodium citrate 1 per cent. solution, and the pressure in the manometer is raised to about the arterial pressure. The vagus nerve is exposed, ligatured in two places, and divided between the ligatures. The depressor nerve is exposed, ligatured, and divided

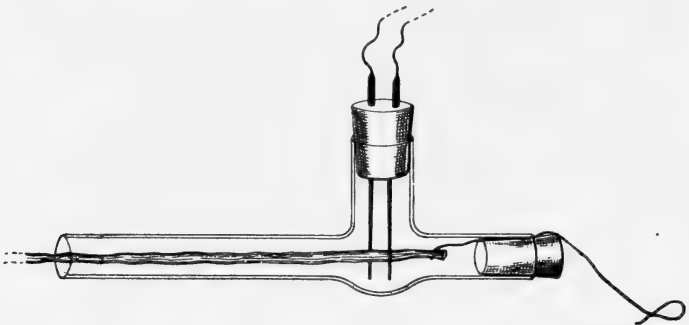


FIG. 152.—Electrodes for exciting vagus and other nerves. (Sherrington.)

below the ligature. The depressor in the cat runs separately from the vagus on the left side. On the right side it can generally be separated from the rest of the vagus without much difficulty. In

the rabbit the depressor runs separately on both sides. In the dog it is bound up in the vago-sympathetic trunk.

The trachea is opened and a tracheal cannula inserted. This is connected by a side tube with a recording tambour. The writing styles of the manometer float and of the tambour are brought to

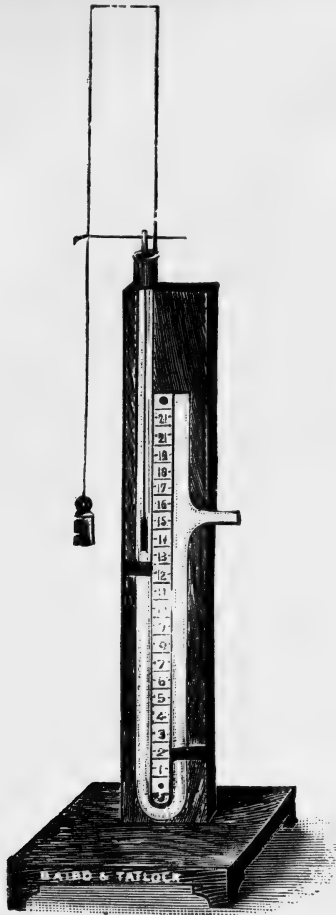


FIG. 153.—Mercurial manometer fitted with float and writing style.

write on the kymograph exactly beneath one another. A clock marking seconds and an electric signal placed in the primary circuit are also brought to write on the kymograph. The primary circuit is arranged to give tetanising shocks, and shielded electrodes are connected with the secondary coil by means of a Du Bois key, and are placed in position under the peripheral end of the vagus nerve.

The clip is then removed from the carotid artery and the kymograph started. Note the height of the arterial pressure, the cardiac pulsations, and the respiratory oscillations of arterial pressure. The pulsations are distorted by the momentum of the mercury.

The inspiratory fall of intra-thoracic pressure aspirates blood into the intra-thoracic veins and thin-walled auricles, and dilates the pulmonary vessels. The descent of the diaphragm expresses blood from the liver and abdominal vessels into the right heart in



FIG. 154.—The kymograph.

the living animal. Thoracic and abdominal breathing have a contrary effect. Thoracic breathing produces an inspiratory fall of arterial pressure, and abdominal an inspiratory rise.

Stimulate the peripheral end of the vagus nerve. The heart is inhibited, and the arterial pressure falls. The heart soon escapes from vagus arrest if the blood pressure is high. The pressure (after vagus inhibition) for a brief space of time rises to a higher level.

The electrodes are now transferred to the central end of the vagus.

Excitation produces either a slight rise (pressor effect) or a slight fall (depressor effect) of pressure. The heart rate is reflexly slowed, and the respiration is stopped with the diaphragm in inspiratory spasm.

The electrodes are next transferred to the central end of the depressor nerve. On excitation the blood-pressure slowly falls, and remains at a lower level so long as the excitation is maintained. The rhythm of the heart is as a rule unaffected. The second vagus

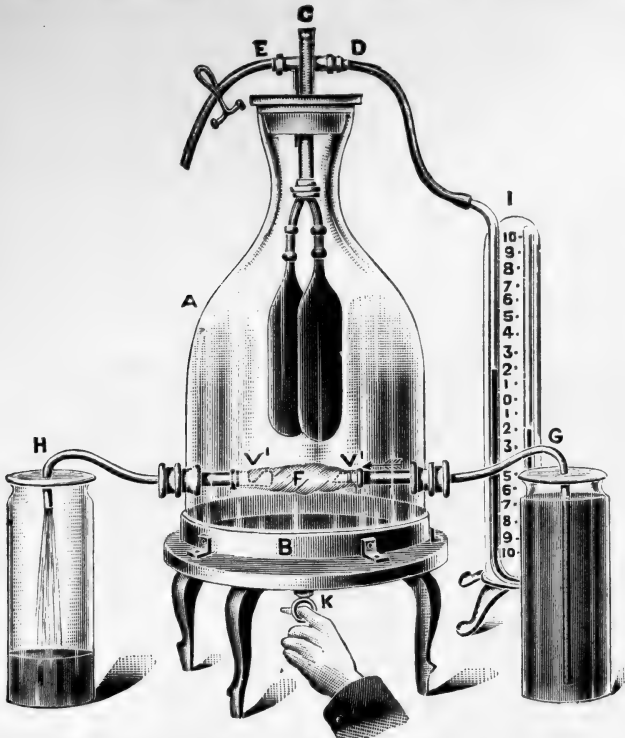


FIG. 155.—Hering's apparatus for demonstrating the action of the respiratory pump.

A, Glass bell, thorax; B, air-tight base; K, diaphragm; C, trachea leading to lungs; I, manometer; E, tube opening into A; F, heart with valves V. The action of the diaphragm pumps air in and out of the lungs and water through the heart. The lungs and heart are thin rubber bags.

nerve is now exposed and divided. The heart beats more rapidly, and the arterial pressure rises. The vagus centre tonically controls the rhythm of the heart.

**Asphyxia.**—The trachea is clamped. Note the sequence of events.

1st stage: Respirations deeper and more ample; the beating of the heart is accelerated and more forcible.

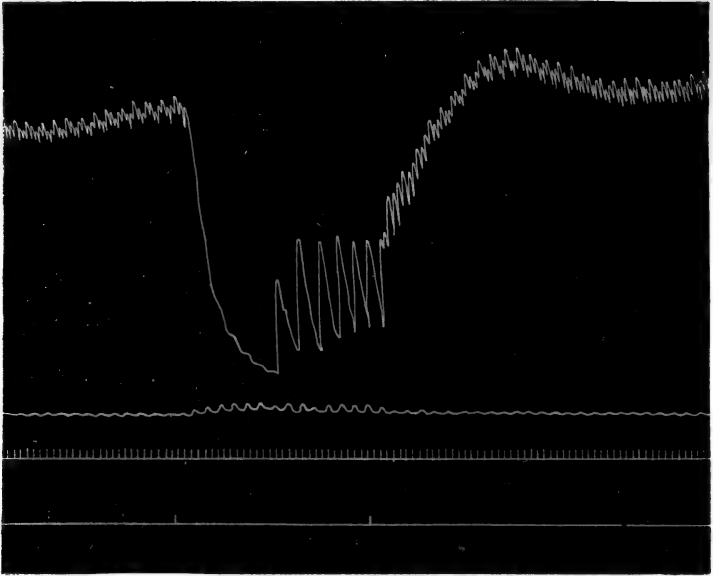


FIG. 156.—The effect of excitation of the peripheral end of the vagus nerve upon the blood pressure in the aorta (top curve) and the vena cava (second curve) of a curarised animal with artificial respiration. Note the inhibition of the heart; the great fall of aortic and the insignificant rise of vena cava pressure; the escape of the heart from the vagus action and the after effect on the aortic pressure.

The time is marked in seconds, and the signal line shows the duration of vagus stimulation. (L.H.)

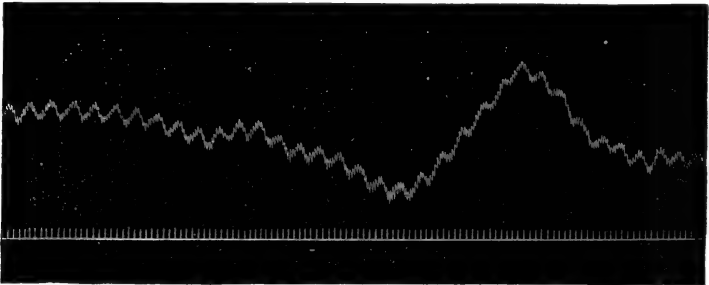


FIG. 157.—Aortic blood pressure.

A, Effect of exciting the central end of vagus. The effect was depressor. B, On shifting up the electrodes to a fresh unexposed part of the nerve the effect changed to pressor. The time is marked in seconds. (L.H.)

2nd stage : Respiration convulsive, less frequent ; blood pressure rising ; heart slow. At the end of the second stage the pupils

dilate and emission of urine and fæces takes place. The veins are congested with black blood.

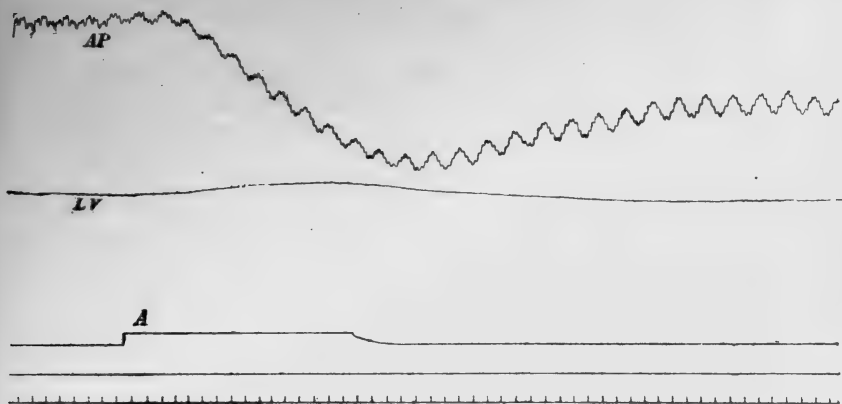


FIG. 158.—Record of arterial pressure (*AP*) and plethysmogram of limb (volume record *LV*). Excitation of the depressor nerve at signal *A*. The limb expanded in spite of the fall of arterial pressure.

The time is marked in seconds. (Bayliss.)

3rd stage: The inspirations, which have occurred at longer and longer intervals, finally cease. The heart beats slowly and with

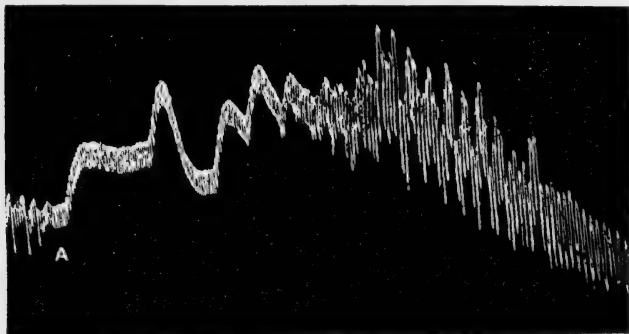


FIG. 159.—Arterial pressure; effect of asphyxia. Animal anæsthetised and curarised. At *A* the artificial respiration was stopped. The large oscillations are Traube-Hering curves. (L.H.)

great force. Finally the heart beat is accelerated, and the blood pressure falls to zero.

## CHAPTER XXXIV

## ANIMAL HEAT

**Difference between Warm-blooded and Cold-blooded Animals.**—Warm-blooded animals, such as mammals and birds, regulate their bodily heat so that their internal temperature remains constant notwithstanding changes in the temperature of their environment; there is little or no difference in the internal temperature of men whether they be living in the tropics or in the arctic regions. Cold-blooded animals cannot regulate their bodily heat; their internal temperature varies with and in the same direction as that of their surroundings. There is, however, no hard and fast distinction between the warm-blooded and the cold-blooded animals. Hibernating mammals, such as the hedgehog, dormouse, and bat, are warm-blooded during the time of activity, but become cold-blooded when they hibernate. Young mammals and birds in a natural condition of immaturity, when they are naked and blind, cannot maintain their temperature at a constant level; they need the warmth of the parent's body. A similar condition is seen in delicate or premature infants.

**The Temperature of Man.**—The average temperature of man is 98.4° F. (36.89° C.). It is taken by means of a clinical thermometer which is either inserted in the rectum, axilla, or mouth, or the subject micturates over the bulb of the thermometer. Take the temperature of your body at each hour of the day. Chart out the results on a temperature chart and observe the daily variation (Fig. 160). Take the temperature before and immediately after muscular exercise, such as a fifteen minutes' run. The temperature may rise to 100°–101° F. (37.78°–38.33° C.) or even more on a hot day. A rise of temperature can be constantly observed if the thermometer be placed in the rectum or stream of urine; the buccal temperature may for the reasons given below show a *fall* in temperature during muscular work. It is important to remember that the daily range in the internal temperature of a healthy man may be from 97.0° F. (36.1° C.) to 99.6° F. (37.56° C.); and that observations taken in the mouth, even when it is firmly closed, are liable to be low, owing to the danger of cooling of the tissues of the mouth, externally by cold air, internally by the inspired air.

**Heat Regulation.**—Take a large frog, and insert a small thermometer in the rectum or flex up the thigh, and insert the thermometer between it and the abdomen, and record its temperature. Place the frog in warm water at 30° C. After ten minutes observe its temperature. It will have reached the same temperature as the water. Cool the frog again in cold water and take its temperature again. Then place it for ten minutes in a thermostat heated to 35° C. In the dry warm air the frog's temperature will not



rise to more than about 30°-33° C. This is owing to the evaporation of water from the frog's skin. Take the temperature of a mouse in the rectum and then place it in a dry thermostat at 30° C. for ten minutes. The temperature of the animal will scarcely vary. Note the quickened respiration of the animal. This increases the evaporation of water from the lungs. Note the way it sprawls out its limbs so as to increase the loss of heat by radiation, convection, and conduction. A man cannot bear for more than a few minutes immersion in a bath of water at a temperature of 44° C., but he can stay for twenty minutes in a dry atmosphere heated to 121° C. The body temperature is then regulated by sweating.

**Loss of Heat.**—An approximate estimation of the amount of moisture lost by a man during exercise or exposure to heat can be made by weighing him naked before and after the exercise. Moisture is lost from the skin and lungs, chiefly from the former.

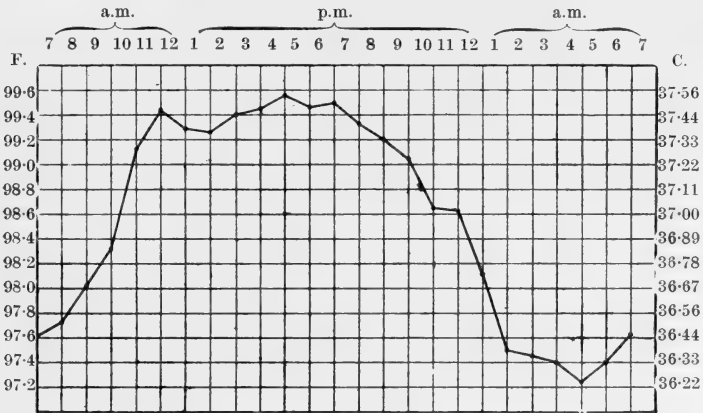


FIG. 160.—Daily variation of temperature (urine) of man. (M.S.P.)

The temperature of the skin also influences the loss of heat by radiation, convection and conduction. It may be readily taken by a mercurial thermometer with a flat bulb. A difference of 10° C. may be observed in the temperature of the skin of the hand in summer and winter; in warm weather the cutaneous blood-vessels are dilated, in cold weather they are contracted. The temperature, however, of those parts of the body which are constantly covered with clothing shows little change.

Clothes diminish the loss of heat from the body by enclosing layers of stationary air, so that the surface of the trunk and limbs is surrounded by a layer of air nearly as warm as the skin.

**EXPERIMENT.**—Take the temperature of the skin of the hand and compare it with that of the chest or abdomen. Compare also the temperatures recorded in the air space between the coat and waist-

coat, between the waistcoat and shirt, between the shirt and vest, and lastly between the vest and skin. In cold weather it will be found that the temperature of these strata of air shows a progressive rise, so that the air between the vest and the skin is almost as warm as the skin itself.

The heat lost from the skin depends upon the temperature and moisture of the air. The temperature recorded by the wet-bulb thermometer is the important factor; it can be taken by wrapping some moist cotton round the bulb of a thermometer and waving it in the air, but always keeping it upright, so that no mechanical displacement of the mercury may occur.

**Sweat.**—The discharge of sweat is under the control of the nervous system, and a simple experiment will prove the existence of sudorific nerves. A cat is killed by an overdose of ether or chloroform. The sciatic nerve is exposed and stimulated by a strong faradic current; after a short delay beads of sweat will be seen on the pads of the foot. The pad of the opposite leg will serve as a contrast.

**Effect of Anæsthesia on the Temperature of the Body.**—**DEMONSTRATION.**—A small mammal is anæsthetised with chloral or urethane after its rectal temperature has been taken. If the animal be now laid on a table with its limbs spread out, and be exposed to the ordinary temperature of a room, its temperature will fall. This is chiefly due to the cessation of muscular movement and the paralysis of the central nervous system, which regulates the temperature of the body. The same effect follows curarisation; section of the spinal cord in the lower cervical region; and the administration of large doses of alcohol.

Anæsthetised patients must be protected from cold. Drunkards who fall asleep on the roadside on a winter's night are easily "frozen to death."

## CHAPTER XXXV

### THE FUNCTIONS OF THE CENTRAL NERVOUS SYSTEM

**The Action of Strychnine and of Chloroform.**—The cerebrum of a frog is destroyed by means of Spencer Wells' forceps, and then under the skin of the back are injected 10 minims of a saturated solution of strychnine (1 in 6,700). In two or three minutes it will be noticed that the frog cannot readily recover its hind legs after a jump, and soon the reflex excitability of the spinal cord is so augmented that a slight touch or puff of wind upon the skin causes a general spasm of the muscles. Convulsions quickly follow, and the rigid body of the frog rests on the mouth and toes, a position known as *emprostotonus*. This attitude is due to the different strength of the various muscles; all are thrown into contraction,

but the stronger overcome the weaker. The muscles are somewhat relaxed after the spasms, but are again sent into tetanus by the slightest touch applied to the skin.

The *tonic* contractions are followed by prolonged twitches or *clonus*.

If during the stage of convulsions a probe be pushed down the vertebral canal, and thus the spinal cord be destroyed, the convulsions cease at once, showing that the strychnine acts upon the ganglion cells and their dendrites in the spinal cord. (See Fig. 162.)

The action of strychnine should be contrasted with that of chloroform. Under the skin of the back of a frog, whose cerebrum has been destroyed by Spencer Wells' forceps, are injected 5 minims of chloroform. The first effect is one of stimulation, but this stage of excitement is quickly followed by marked inco-ordination and weakness. In about ten minutes there is marked anæsthesia, paralysis, and total absence of reflexes.

If the frog be kept moist in a shallow plate full of water, and covered by a bell jar, it may recover from the effects of the chloroform in about eight or nine hours.



FIG. 161.—Diagram of the frog's brain.

1, Olfactory lobe; 2, cerebrum; 3, pineal gland; 4, thalamencephalon; 5, optic lobe; 6, cerebellum; 7, fourth ventricle and medulla oblongata.

### THE DISCHARGE OF NERVOUS IMPULSES FROM THE CENTRAL NERVOUS SYSTEM

The discharge of nervous impulses by the central nervous system can be investigated in the frog by exciting the nerve cells by means of a drug such as strychnine and recording the resulting incomplete tetanus; or in man by the record of the contraction of a muscle thrown into contraction voluntarily, or involuntarily as in shivering.

(a) **The Incomplete Tetanus produced by Strychnine.**—The cerebral hemispheres of a frog are destroyed by compression with a pair of small pliers or Spencer Wells' forceps, and then the gastrocnemius muscle is prepared with the circulation intact. A piece of string is placed under the gastrocnemius muscle and is then tightly tied round the upper portion of the tibio-fibula and the remaining muscles; the leg is now cut away below the ligature. In this manner hæmorrhage is prevented, the circulation in the muscle is intact, and the muscle is free to move with each contraction. A strong pin is placed through the lower extremity of the femur and is pushed firmly into the cork of the myograph; a piece of moist flannel is pinned down over the body of the frog in order to prevent the

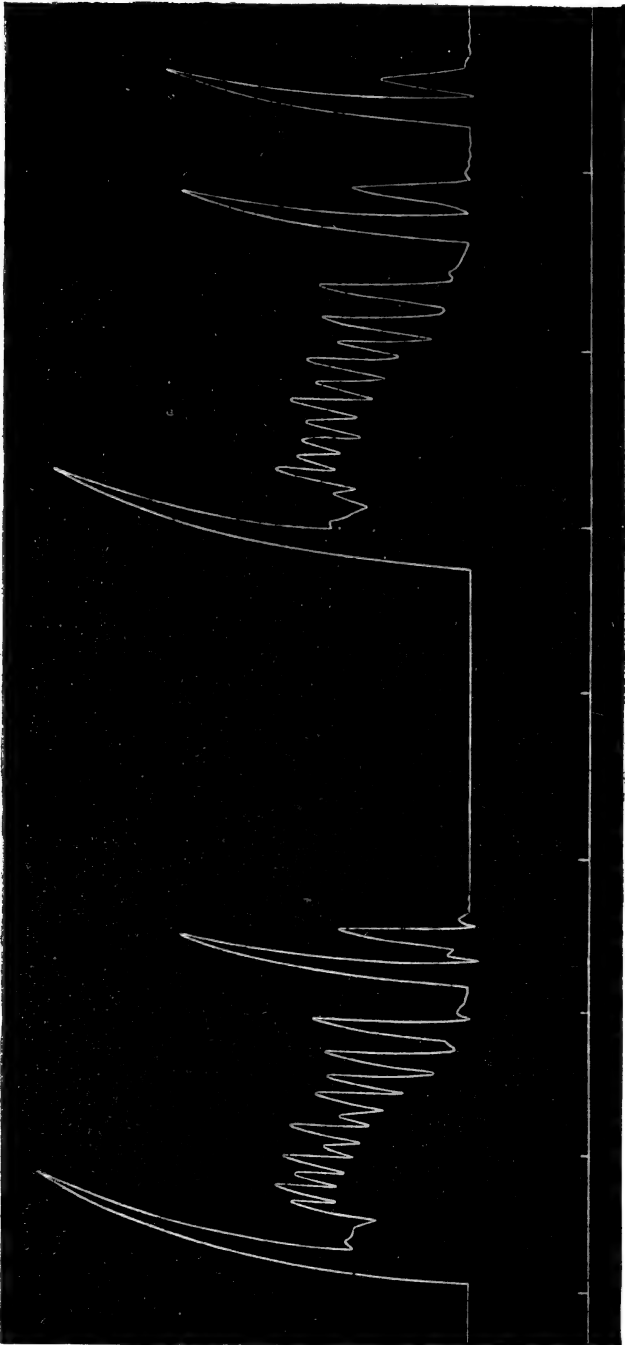


Fig. 162.—Tetanus of the gastrocnemius muscle produced by the action of strychnine upon the spinal cord of a brainless frog. The time is marked in seconds. Temperature of air = 23°. (Pembrey and Phillips.)

contraction of the muscles of the trunk and limbs from disturbing the lever connected with the gastrocnemius muscle.

Strychnine is sparingly soluble in water, 1 in 6,700, but a dose of 10–15 minims (0.592–0.888 c.c.) of a saturated solution of the drug in normal tap-water saline solution will in a frog produce the characteristic convulsions and death. Such a dose is injected under the skin of the frog's back. Twitches and convulsions soon begin and the contractions of the gastrocnemius muscle are recorded simultaneously with the movements of a signal marking seconds (Fig. 162). The number of contractions is about 8 or 10 per second. The stage of incomplete *tetanus* is followed by prolonged twitches or *clonus*. If the spinal cord be destroyed by a probe during the stage of tetanus the contractions will cease at once, showing that the convulsions were due to the action of the drug upon the nerve-cells and dendrites in the spinal end.

#### Record of a Voluntary Contraction.—

If a finger be placed upon a muscle voluntarily thrown into contraction, a series of vibrations can be felt. These can be recorded and their rate determined in the following way.

A receiving tambour, with a button or a piece of cork fixed upon the rubber membrane, is connected with a bellows recorder (Fig. 164) which is arranged to write upon a revolving drum. A chronograph is set up for marking the time in seconds. The button of the tambour is placed upon the adductor pollicis, or the masseter muscle of the subject. When the muscle is voluntarily contracted the lever shows a number of vibrations; these are recorded (Fig. 163). The curve obtained resembles an incomplete tetanus with 6 or 8 vibrations per second; the true rate, as shown by a string galvanometer connected with the muscles, appears to be about 50 per second.

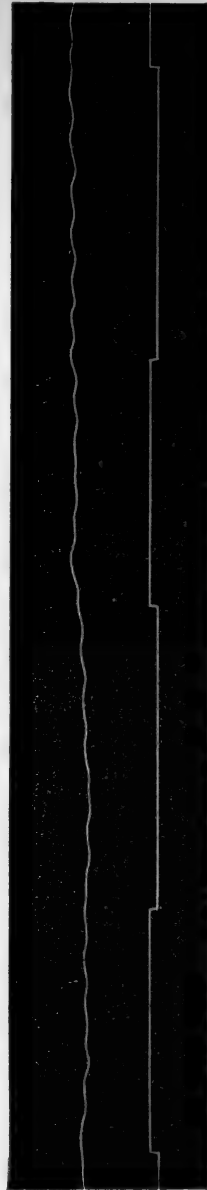


FIG. 163.—Record of a voluntary contraction of the adductor pollicis. The time is marked in seconds.

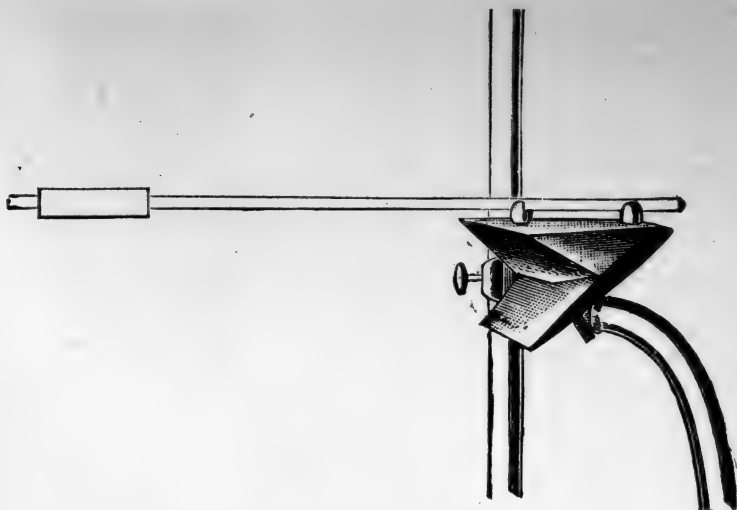


FIG. 164.—Brodie's bellows recorder.  
The bellows are made of aluminium plates and peritoneal membrane.

## CHAPTER XXXVI

### THE FUNCTIONS OF THE ANTERIOR AND POSTERIOR ROOTS OF THE SPINAL CORD. THE MAJENDIE LAW

The researches of Majendie showed that the anterior roots of the spinal cord were motor, and the posterior were sensory; the former nerves are *efferent*, carrying nervous impulses from the spinal cord to the periphery, the latter are *afferent*, carrying impulses from the periphery to the spinal cord. This law can be proved by experiments upon a brainless frog, but careful dissection and manipulation are necessary.

The following are the several stages in the experiment. A small pair of electrodes is made by passing the bared ends of two pieces of fine insulated wire through a piece of cork, and the induction-coil is arranged for single shocks. The cerebrum of a large frog is destroyed by compression with a pair of Spencer Wells' forceps, and then the frog is placed belly-downwards upon a cork board, and is confined to this position by a piece of wet flannel fastened down tightly by pins. A slit is made through the flannel in the line of the vertebral column, and the skin is reflected as far as the end of the urostyle. The ilium is carefully removed on one side, care being taken to avoid cutting any large blood-vessels, for loss of blood would lower the excitability of the spinal cord and obscure the dissection. For a similar reason the medulla oblongata, which contains the vaso-motor centre, was

left intact. After the removal of the ilium the nerves of the sacral plexus can be easily found and followed up to the spinal cord. Starting from the top of the urostyle the laminae of the vertebrae are carefully removed by scissors, the points of which should not be plunged deeply inwards, otherwise the spinal cord will be injured. After the removal of several laminae one of the large nerves of the sacral plexus is followed up to its intervertebral foramen, where a black swelling about the size of the head of a pin will be seen. This is the posterior root-ganglion. It is freed from the foramen by careful dissection, and the roots are traced therefrom to the spinal cord. Fine threads are placed under the roots, which are then divided in the middle of their length by clean sharp scissors.

Stimulation of the peripheral end of the motor root will cause a contraction of the muscles of the corresponding leg; stimulation of the central end with a weak induction shock will cause no movement. On the other hand, stimulation of the peripheral end of the posterior root produces no movement, but a similar stimulus applied to the central end sets up a sensory impulse which produces reflex movements.

The roots of the spinal nerves are longest in the lower segments of the spinal cord; for this reason the experiment is most readily performed in this region. During development the vertebral column grows more quickly than the spinal cord, and thus the lower posterior root ganglia in the intervertebral foramina are separated from the spinal cord by a longer length of nerve-roots than in the case of those supplying the upper limb.

## CHAPTER XXXVII

### INVESTIGATION OF THE MOTOR FUNCTIONS OF THE ALIMENTARY CANAL BY MEANS OF THE X-RAYS

By ARTHUR F. HURST, M.A., M.D., F.R.C.P., Physician, late Demonstrator of Physiology, Guy's Hospital.

The soft viscera are transparent and the salts of the heavy metals are opaque to the X-rays. When therefore any part of the alimentary canal contains food mixed with such a salt, it casts a shadow on the fluorescent screen, when X-rays pass through the body. Barium sulphate is the most useful for this purpose, as it is unaffected by the hydrochloric acid of the gastric juice, and passes through the alimentary canal without influencing its motor functions in any way, and it is much cheaper than the bismuth salts which were formerly used.

A small breakfast should be taken on the morning of the examination in order that the stomach may be as empty as possible when the opaque meal is eaten. Half a pint of bread and milk or porridge mixed with three ounces of barium sulphate forms the meal. A

penny should be fixed over the umbilicus by means of strapping, so that the position of the stomach and intestine in relation to the umbilicus may be recognised. It is unnecessary to take photographs, but the outlines of the shadows seen on the screen should be marked with blue chalk on a superimposed piece of glass, and subsequently copied on to paper.

**Swallowing.**—The examination is begun in the vertical position. A large mouthful of the opaque meal is swallowed, and its passage through the œsophagus into the stomach is watched. For this purpose the rays should pass in an oblique direction through the thorax from the front of the right side to the back of the left side in

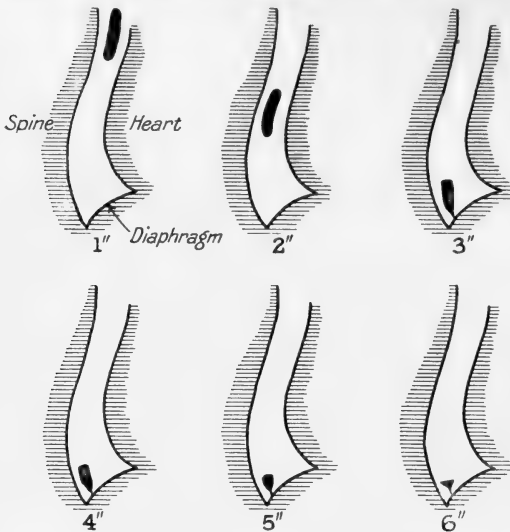


FIG. 165.—Diagrams of position of shadow in œsophagus at intervals of a second after swallowing.

order that nothing should interfere with the view of the œsophagus, which traverses the clear area between the shadow of the heart in front and that of the spine behind. In the vertical position the food passes with great rapidity to the back of the pharynx, and thence equally quickly down the upper part of the œsophagus. A mouthful of ordinary size occupies at any given moment between one and two inches of the

length of the œsophagus. If several mouthfuls are swallowed in rapid succession the whole of the œsophagus becomes visible as a dark shadow. When the fluid reaches the cardia, its rapid progress is arrested owing to the sudden diminution in the lumen of the œsophagus. The lower end of the column of food tapers to a point which represents the cardiac orifice of the stomach, the upper limit becoming horizontal. At a comparatively slow rate the upper horizontal limit of the shadow descends, the lower part remaining unaltered in shape and position until the last trace of the shadow has disappeared. This means that the fluid runs slowly through the narrow cardia into the stomach after having been shot rapidly down the greater part of the œsophagus.

The time which elapses between the initiation of the deglutition



act and the disappearance of the last trace of fluid from the œsophagus should be measured with a stop-watch. It varies between four and nine seconds in different individuals. About one-half of the total period is required for the food to reach the lower end of the œsophagus, the other half being required for its passage through the cardia.

Fig. 165 represents diagrammatically the shadow as seen at intervals of a second.

In the horizontal position the fluid passes along the œsophagus slightly less rapidly than in the vertical position. A similar but more prolonged delay takes place while the food passes through the cardia, the prominent end of the column being in this case rounded. Sometimes a small quantity of the food follows more slowly, and appears as a thin streak instead of the comparatively broad band seen when the œsophagus is filled.

In the inverted position, with the head directed downwards, the food can be seen steadily ascending the œsophagus at about one-third the rate it descends in the vertical position. Owing to its slower passage, the final delay at the cardia is less obvious.

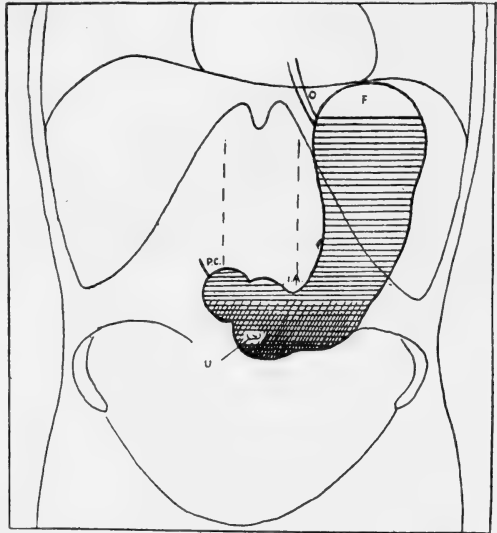


FIG. 166.—Diagram of shadow of normal stomach.

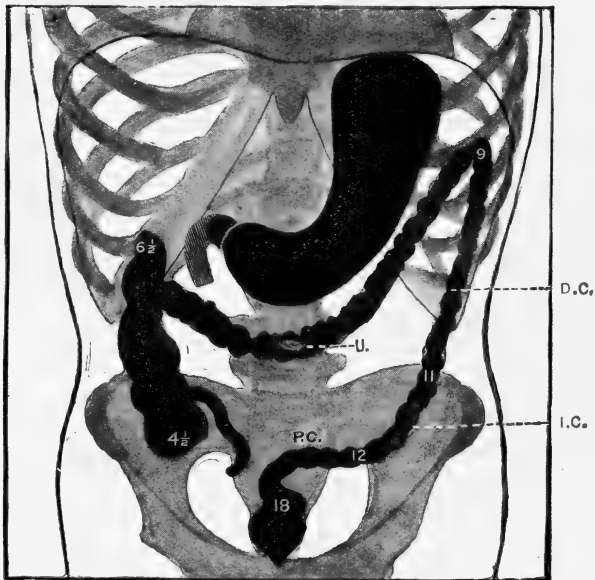
F = fundus ; P.c = pyloric canal ; U = umbilicus.

Sometimes a little fluid runs back from the stomach into the cardiac end of the œsophagus, whence it once again passes into the stomach.

**The Stomach.**—The X-ray tube is now lowered so that the rays may traverse the abdomen, and the individual faces directly forwards. Under the left half of the diaphragm a transparent area is visible, which represents the gas normally present in the fundus of the empty stomach. More of the meal is now swallowed, and it can be seen entering the fundus to the right of this clear area ; the shadow of the stomach becomes gradually more obvious as more of the food is taken. The tone of the stomach diminishes as more food enters, so that the intragastric pressure remains constant. Consequently the upper and lower limits of the shadow remain almost



(a) VERTICAL. b.d., bulbus duodeni.



(b) HORIZONTAL. D.C., descending colon; I.C., iliac colon; P.C., pelvic colon; U., umbilicus.

FIG. 167.—Composite drawings of the alimentary canal to show the average rate in which the food passes through the normal intestines.

The numbers in these figures represent the time after a bismuth or barium meal in which the different parts of the colon were reached. The tracings were made orthodiagraphically and are therefore to scale and not distorted.

(From Hurst's 'Constipation.')

constant, whatever quantity of food is present. When the whole of the meal has been taken, the outline of the stomach should be marked on the screen, together with the position of the umbilicus.

The greater curvature generally reaches a short distance below the level of the umbilicus. The main part of the stomach is almost vertical, and is situated to the left of the middle line. The pyloric end, however, passes upwards and to the right across the middle line (Fig. 166). The upper limit of the gastric contents is situated about  $1\frac{1}{2}$  inches below the diaphragm, and is bounded by a horizontal line, above which is the gas-containing fundus.

On voluntarily contracting the abdominal muscles the lower border of the stomach is raised several inches, and on relaxing them it generally drops an inch or two.

The peristaltic waves can be seen passing from the centre of the greater curvature towards the pylorus. They can, however, be more conveniently studied in the horizontal position.

The examination should be continued in the horizontal position. The greater curvature is now seen to have risen above the umbilicus, and the clear area in the fundus is no longer visible (Fig. 167), the gas having moved to the most superficial part of the stomach, corresponding with which a resonant area can be marked out by percussion below and to the left of the area of cardiac dulness.

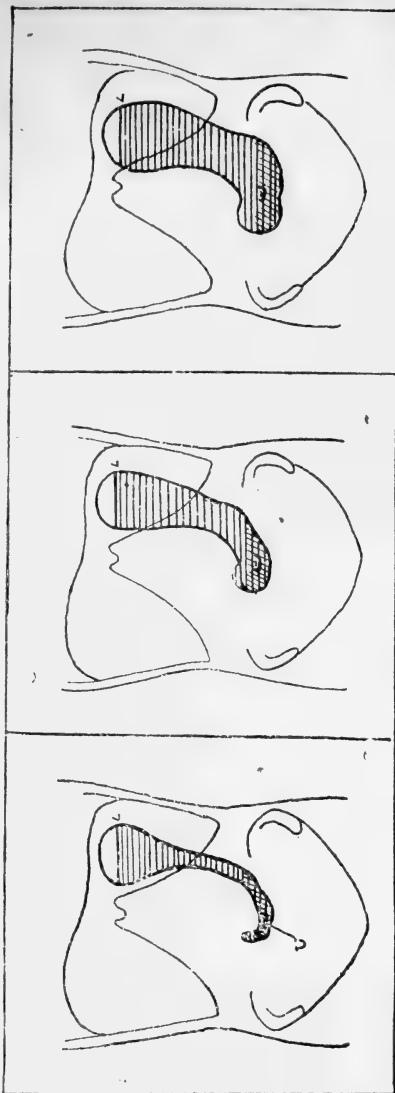


FIG. 168.—Diagrams of shadows of stomach after different quantities of fluid have been swallowed

Peristalsis should now be studied in more detail. The waves start about midway along the greater curvature. As they pass slowly towards the pylorus they become steadily deeper, until about one inch from the entrance into the pyloric canal the extreme pyloric end of the stomach is, as a rule, completely separated from the rest of the organ (Fig. 169). The part thus cut off gradually diminishes in size owing to the further passage of the peristaltic wave and the simultaneous contraction of its longitudinal muscle-fibres. Its contents can be seen to pass partly backwards as a reflux stream into the stomach and partly through the narrow pyloric canal into the duodenum.

**Intestines.**—The first part of the duodenum, the “duodenal cap,” can be clearly defined in the erect position. The chyme collects in it

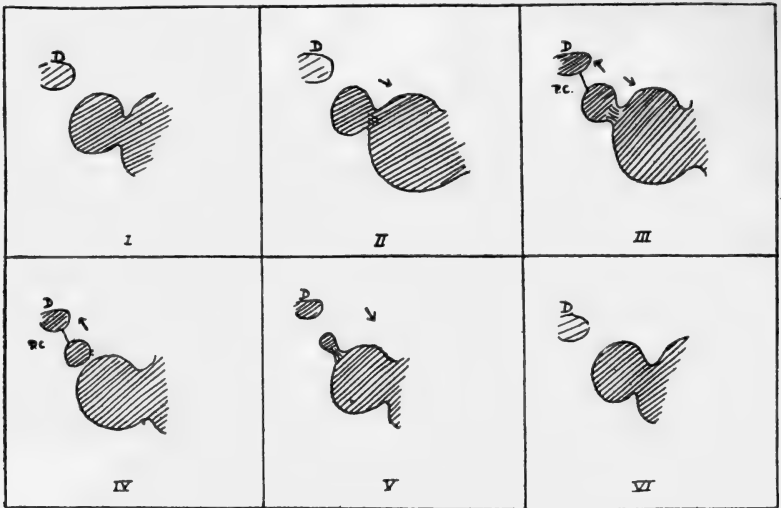


FIG. 169.—Peristalsis in the stomach.

P.C., pyloric canal; D., duodenal cap.

until the next peristaltic wave arrives, and it can then be seen passing very rapidly through the rest of the duodenum into the jejunum.

A second examination should be made between four and five hours after the opaque meal. The stomach is then generally empty. The shadow of the cæcum is seen in the right iliac fossa, and in some individuals a small part of the ascending colon is also visible; the appendix can be frequently recognised, especially if the cæcum and end of the ileum are separated from each other by deep palpation. This examination shows that about four hours are required for the passage of food through the small intestine. A diffuse shadow is generally present at this time in the pelvis. It consists of the terminal coils of the small intestine, the last few inches of which can be recognised as they join the cæcum. With a narrow

diaphragm for the X-rays, short lengths of intestine can generally be clearly defined and their movements studied. A general forward movement of the shadow as a whole, due to peristalsis, can be recognised. At the same time segmentation is seen to occur. The shadow of a short length of intestine, at first of uniform thickness, becomes constricted in its centre. The constriction increases until the single shadow is more or less completely divided into two. Then each half undergoes a similar division, the two central segments of the four produced by the second division joining together. The new central segment then divides again, the segmentation continuing at the rate of about seven divisions a minute. The process is shown diagrammatically in Fig. 170.

A further examination should be made on the following day as early in the morning as convenient. If possible, the bowels should



FIG. 170.—Diagram of segmentation in the human small intestine.  
 (From Hurst's 'Constipation.')

not be opened before this examination. The whole of the large intestine is generally visible, and its position should be marked out in the vertical and in the horizontal position. In the horizontal position the transverse colon is approximately on a level with the umbilicus; in the vertical position it is considerably lower. Both the hepatic and splenic flexures are generally acute, especially in the vertical position, and the two limbs of the flexures may form a single shadow. The effect of straining, as it occurs in defæcation, should be observed: the whole of the colon is greatly depressed, the cæcum and ascending colon together forming a rounded shadow. Peristalsis is very rarely seen, unless examinations are made every quarter of a minute after a meal in order to catch the "mass peristalsis," which occurs after meals as a result of the gastrocolic reflex.

The individual should now retire and open his bowels. On returning a tracing of the colon should again be made. The whole of the large intestine will be seen to have taken part in the act, even the cæcum being less full than it was before. In most cases everything beyond the splenic flexure is evacuated in defæcation (Fig. 171).

The above description refers to an average individual. Very considerable variations occur between different people ; sometimes, for example, the whole of the barium is collected in the pelvic colon at the examination on the second morning, the rest of the colon

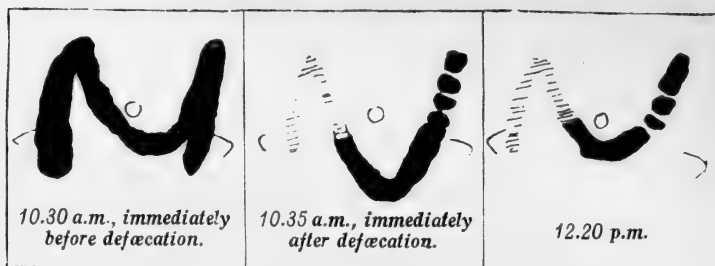


FIG. 171.—Defæcation.

being invisible. Remarkable variations in the shape, size, and position of the different parts of the alimentary canal are also observed in perfectly normal individuals.

Care should be taken to expose the body, and especially the hands and the testicles, to the rays for as short a time as possible. So long as no part of the body is subjected to the direct action of the rays for more than ten minutes during the three examinations, it is unnecessary to wear any special protective covering.







CHEMICAL PHYSIOLOGY



## INTRODUCTION

Chemical physiology is mainly concerned with the materials of which the tissues are composed and the results of the metabolic changes which these materials and also ingested foodstuffs undergo. As Haldane pointed out in his interesting book, *Mechanism, Life and Personality*, "Biology deals at every point with phenomena which, when we examine them, can be resolved into metabolic phenomena—exchange of material and energy, as exemplified in growth, development, maintenance, secretion and absorption, respiration, gross movements in response to stimuli and other excitatory processes." But to quote Haldane again: "We must not mistake measurements of the balance of matter or energy entering and leaving the body, for information as to the manner in which this stream passes through the living tissues."

Still a great deal of information is now available as regards the ultimate fate and in part of the intermediate fate of specific substances ingested. Before however a proper appreciation of the intricacies of the numerous metabolic phenomena, both of the anabolic or synthetic type or of the catabolic or disintegrative type can be obtained, it is necessary that the chemical properties of the materials which compose the living tissue and the nature of the various tissues themselves be studied.

It is true but regrettable that many of the tests and reactions of chemical physiology do not give the sharp end points and definite results to which the student has become accustomed in ordinary chemistry. This is largely due to the fact that the majority of the materials which are dealt with belong to that highly complex series of substances known as the colloids. Further, the student has to grasp the fact that the mere elementary composition, i.e. the content of the substance in Carbon, Hydrogen, Oxygen, Nitrogen, etc., gives little or no information of value when applied to the colloidal materials as found in the tissues where they rarely exist in a pure state. Although the word protoplasm is often used as being almost synonymous with protein, this is not the case. True, it is perhaps probable that in the majority of instances protein is the predominant substance in the protoplasmic mass of the cell, yet intimately incorporated with the protein are found carbohydrates and fatty substances.

In the following pages the subject is dealt with, so far as is possible, from the physiological aspect. Those who desire further information as regards the chemical aspects of the various substances will find the matter dealt with in *Practical Organic and Biochemistry*, by R. H. A. Plimmer.

## CHAPTER I

### PRELIMINARY EXAMINATION

Much time may be saved in the determination of unknown substances if a preliminary examination be carried out.

#### Physical Characters

A. If a fluid, note (1) the colour, (2) transparency, (3) odour, (4) viscosity, (5) specific gravity, and (6) reaction. If a deposit be present or if the fluid be opaque, an examination with the microscope should be carried out.

B. If a solid, note (1) the colour, (2) form—amorphous, crystalline, fibrous, etc., (3) consistence—tough, elastic, hard, soft, etc., (4) odour, (5) solubility in various media: (a) cold, (b) hot—(1) water, (2) 5 per cent. sodium chloride solution, (3) dilute sodium hydrate, (4) dilute mineral acid (HCl), (5) alcohol, (6) ether (*take care no free flame is near*).

#### Chemical Characters

Burn a portion of the substance, or residue after evaporation of a fluid, on a piece of platinum foil. Note (1) changes in substance, (2) odour, (3) ash, if any.

Test for the presence of *nitrogen*, *sulphur* and *phosphorus*.

Into a small hard glass test tube, or a small length of hard glass tubing fused at one end, place a very small portion of the finely-powdered substance to be tested, then add fragments of clean metallic sodium amounting in all to the size of a pea, and another small portion of the substance. Heat at first gently, then strongly until the glass is bright red in colour. Cool and when nearly cold break into a dry evaporating basin under a piece of wet filter paper. After a few minutes add about 15–20 c.c. water, heat to boiling, then filter. Test filtrate.

**Nitrogen.**—To 5 c.c. add a few drops of 0.5 per cent. ferrous sulphate and one or two drops 0.5 per cent. ferric chloride. Boil, then cool and acidify with concentrated HCl. If nitrogen be present a blue solution containing particles of Prussian blue is obtained.

Nitrogen may also be detected by the soda lime test, which is perhaps more easily carried out, but is not quite so reliable as the metallic sodium reaction (Lassaigne's test).

Mix a small quantity of the substance with many times (at least 20) its weight of soda lime in a dry test tube and heat strongly. Test the vapours formed for ammonia by (a) red litmus paper, (b) a glass rod moistened with hydrochloric acid, (c) smell.

**Sulphur.**—To another portion of the filtrate add a few drops of a freshly prepared solution of sodium nitro-prusside. If sulphur be present solution assumes a reddish violet colour.

The presence of sulphur can also be shown by adding a few drops of concentrated sulphuric acid to a portion of the filtrate, place a piece of filter paper moistened with lead acetate solution across the mouth of the test tube and heat. If sulphur be present black lead sulphide is formed.

**Phosphorus.**—To another portion of the filtrate add nitric acid, until markedly acid, then a few drops of ammonium molybdate solution. If phosphorus be present a yellow precipitate of ammonium phospho-molybdate will form on standing.

The presence of **halogens** may also be detected in the filtrate by the ordinary methods.

Tests for **carbon** and **hydrogen** can also be carried out by heating the substance mixed with at least ten times its own weight of *dry* cupric oxide in a hard glass test tube fitted with a rubber cork carrying a length of glass tubing. Tubing is led to a bottle containing baryta water. Heat the test tube containing the mixture, small drops of water will condense in the upper colder part of the test tube and glass tubing and a precipitate of barium carbonate will form in the baryta water.

A general test for **carbohydrates** should also be carried out. Molisch's furfurol test with  $\alpha$ -naphthol is too delicate for general use. Ordinary hydrolysis with a strong mineral acid and a reduction test is preferable. Hydrolyse a small portion of the substance suspended in water by boiling, after the addition of 1-2 c.c. of sulphuric or hydrochloric acid, for five minutes. Cool under the tap. Add a drop or two of copper sulphate solution, then strong sodium hydrate solution cautiously and with frequent shaking until a clear blue solution results. Boil. If carbohydrate be present reduction will take place.

The only reliable general test for the presence of **fat** is extraction with ether by grinding up a portion of the material in a porcelain basin with small amounts of ether, filtering the ethereal extracts and then evaporating. This must be done without the use of a free flame; an electric hot plate serves admirably.

### Choice of Methods

As regards quantitative methods it may be stated, speaking generally, that a gravimetric method is to be preferred to a volu-

metric one and a volumetric to a colorimetric one. The chances of error in the comparing of colours are increased by alterations in the quality of the illumination, eye strain, depth and quality of the colour. The advantages are that these colorimetric methods serve for the detection, very frequently, of minute amounts of the reactive substance, and also of substances in which gravimetric methods are well-nigh useless owing to the small yield.

The same objection, although to a lesser degree, applies to volumetric methods. Here, as a rule, a single colour indicator is adhered to, but it is seldom that one compares the tint obtained with a standard colour. Further variations in the amount of indicator added are apt to occur, with, as a result, variations in the final colour value. Indicators also vary considerably as to their end points (see p. 338). Volumetric methods are however invaluable and extraordinarily close duplicate analyses can be carried out, provided ordinary care and accuracy is employed in the reading of pipettes, burettes, etc.

One of the great assets in gravimetric work is that the final weighing can be carried out with great accuracy and can be checked if necessary. The difficulty here is loss of material due either to solubility or careless handling during filtration.

As regards the statement of results the number of digits given should just be sufficient to do justice to the experiment. Although it would be a matter of little or no importance in an average metabolic experiment whether the total nitrogen output was stated, let us say, as 15.78 or 15.80, still in the earlier stages of the calculation when 2 to 5 c.c. of urine are analysed the results to the third or even to the fourth place are of value so that accumulated rejection errors may be avoided.

In rejecting figures the rule is to increase by one the last figure retained when the rejected figure is 5 or greater; otherwise leave it unchanged. Thus 5.437 would become 5.44, but 5.434 would remain as 5.43.

## CHAPTER II

### THE PROTEINS

The proteins are a group of substances of first-rate importance to the organism as they are essential constituents in every cell and as foodstuffs they are the main source of nitrogen for the building of new tissue. Most of the members of the group are amorphous substances of high molecular weight. The molecule is made up of the elements carbon, hydrogen, nitrogen, oxygen and sulphur. The percentage amount of these various elements differs in different proteins.

	Per cent. present.	
	Variation.	Average.
Carbon . . . . .	50.6—54.5	52
Hydrogen . . . . .	6.5— 7.3	7
Oxygen . . . . .	15.0—17.6	16
Nitrogen . . . . .	21.5—23.5	24
Sulphur . . . . .	0.3— 2.2	1

Proteins have been divided into a number of classes in a provisional manner as our present knowledge of their chemical structure does not permit of a strictly chemical classification. The arrangement which has met with most general acceptance is as follows :

- (1) Protamines.
- (2) Histones.
- (3) Albumins
- (4) Globulins
- (5) Glutelins
- (6) Gliadins (Prolamines)
- (7) Sclero-proteins (Albuminoids).
- (8) Phospho-proteins.
- (9) Conjugated proteins.
  - (a) Gluco-proteins (Glyco-proteins).
  - (b) Nucleo-proteins.
  - (c) Chromo-proteins.
- (10) Derivatives of proteins (Hydrolysed proteins).

These proteins have one thing in common, namely, that when completely hydrolysed by mineral acids, alkalis or ferments, they yield a variety of amino acids. During this process of hydrolysis the large molecule of the protein is broken down and water is taken up.

The resulting amino acids, which vary, both in number and amount, in the different proteins, are simple combinations in which a hydrogen of a fatty acid is replaced by an amino group ( $\text{NH}_2$ ); thus, for example, propionic acid is  $\text{CH}_3\text{CH}_2\text{COOH}$ ; if now one of the hydrogens is replaced by  $\text{NH}_2$  we get alanine or amino propionic acid,  $\text{CH}_3\text{CH}(\text{NH}_2)\text{COOH}$ . The great majority of the amino acids found in the tissues have a hydrogen atom attached to the carbon atom nearest the carboxyl group replaced by the  $\text{NH}_2$  and they are therefore  $\alpha$ -amino acids. Further, some of the amino acids in place of having a single  $\text{NH}_2$  group attached have two. The amino acids present may be divided into three classes.

#### I. Monoamino acids :

- (a) with one carboxyl group : glycine, alanine, leucine, etc.
- (b) with two carboxyl groups : aspartic and glutamic acids.

(c) of the aromatic series : tyrosine, phenylalanine.

#### II. Diamino acids : Lysine, arginine, (cystine), etc.

#### III. Heterocyclic amino acids : histidine, tryptophan, etc.



Or, alternatively, they may simply be classified as neutral, acid or basic ampholytes, i.e. substances which can either function as acids or bases. In this classification to the neutral group belong the monoamino monocarboxylic acids including tyrosine, phenylalanine, tryptophan and cystine, to the acid group the monoamino dicarboxylic acids and to the basic the diamino acids and histidine.

The following table gives some idea of the percentage distribution of the various amino acids in different proteins :

	Egg Albumin.	Serum Albumin.	Serum Globulin.	Caseinogen (Cow).	Gelatine.
Glycine . . . . .	0	0	3.5	0	16.5
Alanine . . . . .	2.1	2.7	2.2	0.9	0.8
Leucine . . . . .	6.1	20.0	18.7	10.5	2.1
Aspartic acid . . . . .	1.5	3.1	2.5	1.2	0.6
Glutamic acid . . . . .	8.0	7.7	8.5	11.0	0.9
Cystine . . . . .	0.2	2.5	0.7	0.1	—
Histidine . . . . .	—	—	—	2.6	—
Lysine . . . . .	—	—	—	5.8	2.8
Arginine . . . . .	—	—	—	4.8	7.6
Phenylalanine . . . . .	4.4	3.1	3.8	3.2	0.4
Tyrosine . . . . .	1.1	2.1	2.5	4.5	0
Tryptophan . . . . .	+	+	+	1.5	0

### The Physical and Chemical Properties of Proteins

I. **Solubility.**—Most proteins<sup>1</sup> are insoluble in alcohol and ether. They vary as to their solubility in water; of the more common proteins, albumins are soluble in water and globulins in weak saline solutions. Some, however, are not soluble, even in concentrated saline solutions.

EXPERIMENT. From the undiluted egg-white provided prepare a solution of egg albumin by adding 10 volumes of distilled water and mixing thoroughly in a flask. An opalescent solution is thus obtained, the opalescence being partly due to the colloidal nature of the solution, although in part to some other protein (ovo-mucin), which has not gone into solution. This can be removed by filtering through fine muslin. Note that this solution, like all colloidal solutions, *gives a persistent froth on shaking*.

The solution prepared above can be used in the subsequent experiments, unless otherwise stated.

II. **Diffusibility.**—As the proteins give only *colloidal solutions*, these solutions will not dialyse, that is, diffuse through animal membranes or parchment paper. In this they are unlike *crystalloids*, such as inorganic salts, which readily diffuse through such membranes. Of the various forms of dialyser, a tube of parchment is the simplest.

EXPERIMENT. Place a mixture of diluted blood and of a 10 per

<sup>1</sup> Some vegetable proteins are soluble in alcohol.

cent. sodium chloride solution in the dialyser provided. Test a sample of distilled water with silver nitrate, and note that no haze of silver chloride occurs. Place the dialyser in a beaker of this water and allow dialysis to proceed for a day. On now testing the water in the beaker for chlorides with silver nitrate, it will be found that a white precipitate of silver chloride occurs, showing that the chlorides have diffused through the parchment. It can be shown, however, that no protein has dialysed through, *by the absence of pigment* and by applying the tests for protein given below.

III. **Heat Coagulation.**—Most of the so-called native proteins (albumins and globulins) coagulate when their solutions are heated.

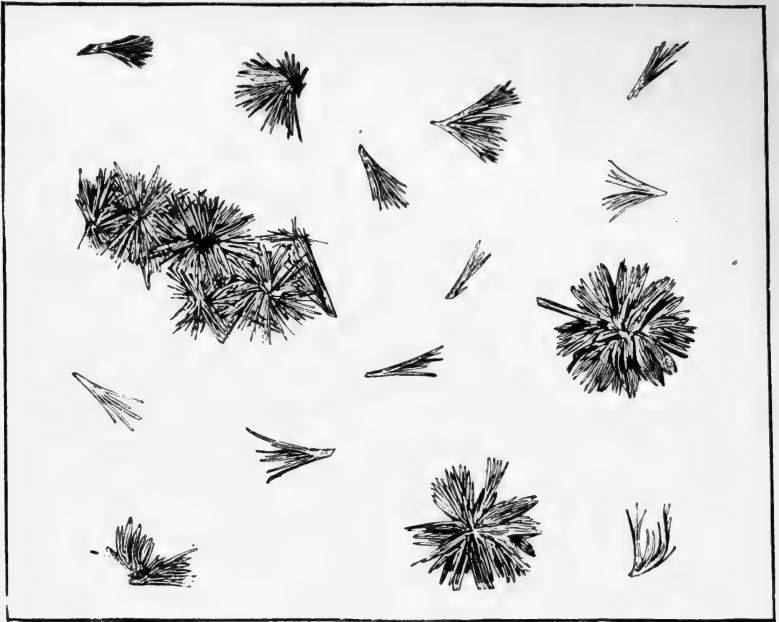


FIG. 172.—Crystallised albumin.  $\times 600$ .

Different proteins coagulate at different temperatures, varying usually from  $56^{\circ}\text{C.}$ – $78^{\circ}\text{C.}$  A faint degree of acidity and the presence of a neutral salt greatly favours heat coagulation.

EXPERIMENT. Fill a narrow glass tube with some egg-white solution, faintly acidulated with acetic acid, and seal off one end. Now fix this to the lower end of the thermometer by means of small elastic bands. Gradually heat in a test tube placed in a water bath and observe the temperature at which the albumin becomes opaque and set.

IV. **Crystallisation.**—Most proteins crystallise with difficulty; the blood pigment of certain animals, however, crystallises readily. (See later under Blood, Chapter XI.) Egg albumin and serum

albumin have, however, been crystallised. Certain vegetable proteins, e.g. the globulin of hemp seed (edestin), crystallise more easily.

Extract hemp seed, which has been thoroughly pounded, with warm 5 per cent. sodium chloride (50° C.) and place extract in a dialyser overnight. As the result of dialysis, crystals of edestin become deposited in the tube. Examine those placed under the microscope. Crystals of edestin may also be obtained, on standing, by cooling the extract of hemp seed with ice.

To obtain crystals of egg albumin the whites of several eggs are mixed with an exactly equal amount of a fully saturated solution of ammonium sulphate. This precipitates the globulins. The ammonium sulphate solution must be exactly neutral in reaction and should be added to the egg-white in small quantities at a time, the mixture being briskly stirred between each addition. The precipitated globulin is filtered off, and the filtrate, which reacts alkaline to litmus, is treated with ammonium sulphate, drop by drop, until a faint haze of precipitated albumin is obtained. A drop of water is added, so that the haze just disappears. The solution is now treated with 10 per cent. acetic acid, drop by drop, until a precipitate of albumin just forms. The flask is set aside; in about twenty hours it will be found that a large number of needle shaped crystals have become deposited (see Fig. 172).

**V. Rotation of Light.**—All proteins are lævo-rotatory. Some combined proteins, such as hæmoglobin and nucleo-protein, are dextro-rotatory, but their protein portion is lævo-rotatory.

**VI. Electrical Properties.**—Although crystalloid free protein solutions are practically non-conductors, it is found that when placed in an electric field proteins differ in their reaction. Some migrate towards the positive pole, some towards the negative and some show no definite direction; there are therefore protein cations and anions. The third class are uncharged or isoelectric. Proteins of opposite charges mutually precipitate one another.

**VII. Colour Reactions.**—This group of reactions is very important, as each reaction yields information as to the constitution of the protein molecule. The meaning of each test should therefore be carefully noted.

(a) **The Biuret Reaction** (Piotrowski's test).

**EXPERIMENT.** Pour a drop of weak copper sulphate into a test tube. Now add some 20 per cent. caustic soda until a pale blue colour is obtained (about 15 c.c.). Divide this into three portions, A, B, C. Keep A as control colour. To B add a few drops of diluted egg-white. To C add the same number of drops of the commercial peptone provided. Note the violet colour with albumin, the pink colour with the peptone solution. If too much copper has been added filtration often gets rid of the excess copper hydrate.

It is important to keep control tube A, since in using very weak solutions a slight change in colour can be detected by comparison with the control.

All proteins give either a purple or pink colour with this test. It shows that the protein contains two or more CO — NH — groups linked together. The same reaction is given by the substance biuret which is formed when urea is heated, hence the name.

**(b) Xanthoproteic Reaction.**

**EXPERIMENT.** To about 5 c.c. of the solution of egg-white add a few drops of strong nitric acid; a white precipitate results. Warm this and the precipitate changes to a yellow curd. If little protein is present no visible coagulation takes place. Cool under the tap. Add a few drops of strong ammonia; the yellow colour changes to a brilliant orange. The name xanthoproteic (*yellow* protein) will help the student to remember the colour obtained. This test shows the presence of the benzene ring in the protein molecule; hence only proteins containing such a ring give this test.

**(c) Millon's Reaction.**

**EXPERIMENT.** Add a few drops of Millon's reagent (which consists of a solution of mercurous and mercuric nitrates) to some of the egg-white solution. A white coagulum forms, which on warming changes to a brick-red curd. With some substances no precipitation takes place but solution becomes red on heating.

This reaction shows the presence of a benzene ring with an hydroxyl group attached to it, *i.e.*, of the phenolic group such as occurs in tyrosine.

**(d) The Glyoxylic Acid Test (Hopkins' modification of Adamkiewicz's Reaction).**

**EXPERIMENT.** To some egg-white solution in a test tube add about 1 c.c. of glyoxylic acid solution, and run in carefully without mixing ordinary strong sulphuric acid. A violet ring is obtained at the junction of the fluids, which extends into the supernatant egg-white solution when the tube is gently agitated.

This test depends upon the presence of tryptophan (indol amino-propionic acid) in the protein molecule, and is only given by proteins containing such a grouping.

**(e) The  $\alpha$ -Naphthol Test (Molisch's test). (V, p. 210).**

Proteins containing a carbohydrate moiety yield this test. The purple colour should be very pronounced before the test is deemed positive. The green colour obtained plays no part in the reaction. The test is not very reliable.

**VIII. Precipitation by Neutral Salts ("Salting out").****(A) Ammonium Sulphate.**

**EXPERIMENT.** To some egg-white solution add an *equal* amount of saturated solution of ammonium sulphate = *half saturation*. A white precipitate of globulin is produced. Filter; keep the filtrate. After washing the residue with saturated ammonium sulphate dissolve it in a little water and boil. Note that the protein is coagulated in fine flakes. Divide the filtrate:

(a) Add crystals of  $(\text{NH}_4)_2\text{SO}_4$  in excess (*full saturation*). The albumin is now salted out.

(b) Boil; flakes of coagulated protein show the presence of coagulable protein (albumin). Half saturation with  $(\text{NH}_4)_2\text{SO}_4$  therefore precipitates globulins; full saturation precipitates albumins.

(B) *Magnesium Sulphate.*

EXPERIMENT. Fully saturate (i.e. add crystals) the solution of egg-white with  $MgSO_4$ . A precipitate of globulin results. Filter. Prove by heat coagulation and by fully saturating with  $(NH_4)_2SO_4$  that protein (albumin) is left in the filtrate. Magnesium sulphate in full saturation precipitates globulins, but not albumins.

(C) *Sodium Chloride, Ammonium Chloride.* These salts resemble magnesium sulphate in their "salting out" properties.

(D) *Sodium Sulphate* possesses at  $30^\circ C$ . the same protein precipitating powers as ammonium sulphate. It is of great advantage when it is desired to estimate the amount of protein in any fluid. By precipitating with sodium sulphate and determining the total nitrogen in the precipitate by Kjeldahl's method (see Urine) the amount of protein is found by multiplying by 6.25.

**IX. Coagulants of Proteins.**—A coagulum differs from a precipitate in that it is no longer soluble in its original solvent; in other words, its physical or chemical nature has undergone some change. Such is the case in the coagulation of protein by heat. Other coagulants of protein are:—Mechanical agitation, mineral acids and salts, and other acids such as tannic, picric, etc.

EXPERIMENT I. Violently shake some egg-white solution with sand. Strings of coagulated protein are deposited.

EXPERIMENT II. To some egg-white solution add gently some strong  $HNO_3$ . A white precipitate appears, which is insoluble on heating (cf. Proteoses).

EXPERIMENT III. Acidulate some egg-white solution strongly with acetic acid, then add strong potassium ferrocyanide—a whitish yellow precipitate.

EXPERIMENT IV. Add picric acid—a white precipitate. Many other acids, such as phosphomolybdic, phosphotungstic, trichloroacetic and salicyl sulphonic are used to precipitate proteins.

Alcohol precipitates all proteins. At first it forms a precipitate; but if the action be prolonged this changes to a coagulum. Peptone and fibrin ferment (thrombin) take longer to undergo this change; advantage is taken of this to separate these bodies from other proteins.

### CHAPTER III

#### PROTEINS—Continued

As regards the various groups of proteins they have all more or less special properties.

**Protamines.**—These are usually found in the roe—or milt of fish as salts of nucleic acid. They are strongly basic substances which readily turn litmus blue and take up carbon dioxide from the air. They are soluble in water and are not coagulated on heating. As a rule the only colour reaction given is the biuret. On decom-

position they yield large amounts of the diamino acids, especially arginine.

**Histones** have been obtained from various sources, but globin of blood is the most important of the group. They are strongly basic substances and resemble in their properties in part native protein, in part protamine and in part proteose. Their behaviour to heat depends on the presence or absence of salts; no coagulation takes place when salts are absent.

Biuret, xanthoproteic and Millon's reactions are given, and a large yield of diamino acids, especially histidine, on decomposition.

The group of **native proteins** has been already studied in the preceding experiments with the egg-white solution. The main difference between the albumins and the globulins is that of solubility. It has also been shown chemically that the products of hydrolysis differ, the albumins yielding no glycine. Upon hydrolysis all yield members of the chief amino acid groups.

**Albumins** are soluble in distilled water and in saturated solutions of all neutral salts except ammonium sulphate and anhydrous sodium sulphate, in which they are insoluble. They are, however, soluble in half-saturated solutions of these salts.

**Globulins** are insoluble in distilled water and in saturated solutions of all neutral salts. They are, moreover, insoluble in half-saturated solutions of ammonium sulphate and anhydrous sodium sulphate. They are soluble in weak saline solutions.

The chief kinds of albumins are egg albumin, serum albumin (see Blood), and lactalbumin (see Milk).

The most important globulins are egg globulin, serum globulin, fibrinogen (for both see Blood), and myosinogen (see Muscle).

**Plant Proteins.**—This comprises the two groups (*a*) Glutelins and (*b*) Gliadins (Prolamines), and they form the bulk of the protein present in such cereals as wheat, barley, maize, etc. The so-called gluten of wheat is composed of approximately equal amounts of the two groups. The glutelins are insoluble in water and in dilute neutral salt solution, but are soluble in dilute acids and alkalis. They are coagulable on heating. The gliadins are insoluble in water and in absolute alcohol but dissolve in about 70 per cent. alcohol. They are insoluble in dilute neutral salt solutions. They have been called prolamines because they have been shown to be rich in the amino acid proline (pyrrolidine carboxylic acid) and amide groups. They are also rich in glutamic acid.

**Phosphoproteins.**—The chief members of this group are the caseinogens of milk and the vitellins from egg-yolk. They derive their name from the large amount of phosphorus contained in their molecule. They differ, however, from nucleoproteins in containing *no* purin bases.

Dissolve some commercial caseinogen in 2 per cent. caustic soda, and perform the following experiments:—

- I. Note that it is precipitated with 1 per cent. acetic acid, the precipitate being soluble in excess of acid.
- II. Perform the colour tests for protein, and record your results.
- III. Perform the "salting out" tests with  $(\text{NH}_4)_2\text{SO}_4$  and  $\text{MgSO}_4$ .
- IV. Heat the solution.

With the solid substance perform the following experiments:—

- V. Heat some solid caseinogen upon a piece of broken porcelain with "combustion mixture" (a mixture of sodium carbonate and potassium nitrate). When cool, extract with nitric acid, filter, add ammonium molybdate in nitric acid, and heat. The canary yellow precipitate denotes presence of phosphorus.
- VI. Heat a little caseinogen with 1 per cent.  $\text{NaOH}$  in the incubator or on a water bath at  $37^\circ \text{C}$ . for twenty-four hours. Phosphoric acid is broken off. Precipitate the phosphoric acid, after acidifying with acetic acid and filtering, by the addition of ammoniacal magnesium citrate. Filter. Dissolve the precipitate in nitric acid, and test with molybdate as above.

In connection with the above experiments it will be found that caseinogen yields all the colour tests except Molisch. It therefore contains no carbohydrate group (see p. 210). The xanthoproteic, Millon's, and the glyoxylic tests will be very well marked, showing that caseinogen is rich in tyrosin and tryptophan.

In "salting out" caseinogen behaves like a globulin, being precipitated by full saturation with magnesium sulphate and half saturation of ammonium sulphate.

Caseinogen is *not* coagulated by heat.

The **Sclero-proteins** comprise the group of proteins formerly termed albuminoids. They are obtained mainly from "the hard" or supporting structures of the body. Those of physiological importance are *Collagen*, *Elastin* and the *Keratins*.

**Collagen**, the precursor of gelatin, forms the chief constituent of white fibrous tissue and of the organic substance of bone. It also exists in cartilage, where, however, it is mixed with several other bodies (see under gluco-proteins, p. 201).

**Preparation of Collagen.**—A piece of tendon is macerated overnight in 1 per cent. caustic alkali to remove other proteins, and then washed with water till alkali free. The resulting mass is collagen. Place a piece of this in a flask and boil it for ten minutes with water which is rendered faintly acid with acetic acid. By this treatment, the collagen is transformed into gelatin and, on cooling the solution, it gelatinises. Solutions containing about 1 per cent. and upwards set to a jelly on cooling. If the process of heating and cooling be repeated too often the solution ceases to set.

**Gelatin.**—This is really the hydride of collagen, the boiling with acidulated water in the above experiment having caused the collagen to take up a molecule of water. Conversely, the gelatin can be reconverted into collagen by heating it to  $130^\circ \text{C}$ ., whereby it loses water.

**EXPERIMENT.** Apply the following tests to a solution of gelatin in water: (1) The Biuret reaction: a violet colour is produced.

(2) The xanthoproteic reaction: only a slight coloration is produced. (3) The Millon's test: only a slight reddening of the precipitate occurs on boiling. (4) The glyoxylic test: absent or very faint. (5) Half saturation with  $(\text{NH}_4)_2\text{SO}_4$ : salted out. It is also precipitated on full saturation with magnesium sulphate, also on full saturation with sodium chloride in an acid medium.

The reason why the second, third and fourth tests are not very distinct, is because gelatin does not yield aromatic bodies on decomposition, and both these tests depend on the presence of aromatic bodies. Some varieties of gelatin give these reactions more distinctly than others, but absolutely pure gelatin<sup>1</sup> is said not to give them at all, so that their presence is held to depend on contamination of the gelatin with native protein.

**Elastin.**—This is the chief protein constituent of elastic fibrous tissue. It is quite insoluble in water and neutral salt solutions. By prolonged treatment with dilute acids or alkalis it goes slowly into solution, with stronger solutions it is more rapidly dissolved, but it also undergoes decomposition.

**Keratins.**—These are horny materials found in hair, nails, hoofs and horns, etc. These substances are characterised by their high sulphur content. They are quite insoluble in water and neutral salt solutions. They are also insoluble in dilute acids and alkalis, but on treatment with stronger solutions they go into solution and at the same time are decomposed. They give the ordinary protein colour reactions.

**EXPERIMENT I.** Using small pieces of yellow elastic tissue try its solubility in water, hot and cold, and in strong alkalis and acids. Do the colour reactions.

**EXPERIMENT II.** Using hair try its solubility in water and in strong acids and alkalis. The presence of loosely combined sulphur can readily be shown by heating nails or hair with caustic soda in a test tube. On the cautious addition of strong acid to the cooled alkaline solution  $\text{H}_2\text{S}$  is given off and can be detected by holding paper moistened with lead acetate solution over the mouth of the test tube.

**EXPERIMENT III.** Using small pieces of finger-nail show that keratin also gives the xanthoproteic and Millon tests.

**Conjugated Proteins.**—In this group we have proteins to which groups (called prosthetic groups) other than protein are united to form a complex molecule. The chief groups are:

- (i) The chromo-proteins.
- (ii) The gluco-proteins.
- (iii) The nucleo-proteins.

**Chromo-proteins.**—This group of substances is a combination of protein with a coloured substance. The best known example is, of course, hæmoglobin, a compound of the iron containing substance

<sup>1</sup> Pure gelatin is said to lack Tyrosine, Tryptophan and Cystine.



hæmatin and the histone globin. In certain of the lower forms of life, molluscs for example, there is believed to exist a conjugated protein hæmocyanin in which the pigment substance contains copper in place of iron.

**Glucoproteins.**—This class of protein has as a prosthetic group some form of carbohydrate, very frequently glucosamine. Glucosamine is a sugar which contains an amino group in its molecule.

They are often separated into three groups, mucins, pseudomucins and mucoids. The mucins are soluble in water and dilute alkali, but are precipitated with excess acetic acid. They give viscid solutions. The pseudomucins are equally soluble but are not precipitated by acetic acid. The mucoids are soluble in water and in dilute acids and alkalis. Their solutions are not viscid. Mucins are found in the saliva, sputum and synovial fluid. Pseudomucin is present in fluid from ovarian cysts. Mucoids are found in the cornea, the lens, in cartilage, tendon and bones. Mucoid-like substances can be also obtained from serum. The so-called nubecolæ of urine is also a mucoid. The different glucoproteins yield varying amounts of reducing substance on hydrolysis.

**EXPERIMENT.** Collect some saliva in a test tube, note its viscosity ; add to it a few drops of 1 per cent. acetic acid ; a stringy precipitate of mucin results. It is insoluble in excess of acetic acid. Filter. To residue add a few drops of weak sodium carbonate solution, when the precipitate will dissolve. Test this with protein colour tests, including Molisch. If enough mucin can be collected the presence of carbohydrate can be shown by boiling with a mineral acid, then doing an ordinary reduction test.

**EXPERIMENT.** Mucin can be prepared from connective tissue where it is very abundant, by extracting the latter with a weak alkali (lime water). The mucin is precipitated by a weak acid, and the resulting precipitate then boiled for about ten minutes with hydrochloric acid (1 part concentrated acid + 3 parts water). The resulting solution is cooled and neutralised, and examined for protein and carbohydrate. Divide the solution into portions, *a* and *b*.

To (*a*) apply the Biuret reaction—a violet or pink colour is produced, showing the presence of the protein moiety.

To (*b*) add a drop of copper sulphate solution, and, if necessary, some caustic alkali till a blue solution is obtained. Now boil, when reduction to cuprous oxide will occur, demonstrating the presence of the carbohydrate moiety.

**Nucleo-proteins.**—These are derived for the most part from the nuclein of the cells and are, therefore, widely distributed. The prosthetic group combined with protein is nucleic acid. They may, indeed, be regarded as protein salts of nucleic acid. The two components are readily separated, especially by the action of alkali. Two varieties *a* and *β* nucleo-proteins are recognised. It is questionable if the *β* form is actually a constituent of the cell nucleus.

Nucleo-proteins on digestion with pepsin are not completely broken down. A certain amount of the protein is left in combination with the nucleic acid ; this material is called *nuclein*, i.e. nuclein is the residue left after peptic digestion of a nucleo-protein. Nuclein in its turn may be split into protein and nucleic acid. Nucleic acid, on complete hydrolysis, yields various substances, a carbohydrate, phosphoric acid, two purine and two pyrimidine bases.

It has further been found that animal nucleic acid differs in certain details from plant nucleic acid.

<i>Animal Nucleic Acid.</i>		<i>Plant Nucleic Acid.</i>	
Phosphoric acid		Phosphoric acid	
Carbohydrate = a hexose		Carbohydrate = a pentose	
2 Purine bases	{ Guanine Adenine	2 Purine bases	{ Guanine Adenine
2 Pyrimidine bases	{ Cytosine Thymine	2 Pyrimidine bases	{ Cytosine Uracil

It would seem also to be conclusively proven that there is a combination between the phosphoric acid and the carbohydrate and between the carbohydrate and a purine base. Such compounds of carbohydrate, phosphoric acid and purine base are known as mononucleotides, and as there are four such groupings in nucleic acid it is regarded as a tetranucleotide.

Nucleo-proteins and nucleins are insoluble in water, also in dilute acid solutions, but are soluble in dilute alkalis and strong acids. They can be precipitated from their alkaline solutions by acidification with acetic acid. Phosphoric acid is not liberated on hydrolysis with alkali (differentiation from phospho-proteins, *q.v.*), but it is yielded on hydrolysis with acid.

In order to prepare nucleo-protein the following method may be used :—

A cellular organ, such as the thymus or pancreas, is minced and macerated overnight with water made faintly alkaline with caustic soda or ammonia. The extract is then strained through muslin, litmus added, and then weak acetic acid. When the reaction becomes faintly acid, a copious precipitate of nucleo-protein occurs. The nucleo-protein is filtered off and dissolved in weak alkali (1 per cent. sodium carbonate). (See also p. 325.)

**EXPERIMENT.** Some of this alkaline solution is supplied :

(1) Add acetic acid—white precipitate soluble with difficulty in excess. (Cf. mucin, which is insoluble, and caseinogen, which is readily soluble.)

(2) Perform the protein colour tests.

(3) Ascertain how it is “salted out.”

**Derivatives of Proteins.**—When proteins are hydrolysed either by means of inorganic reagents like hydrochloric acid or by proteolytic ferments a series of changes take place with the formation of increasingly simple substances before the ultimate amino acid or peptide form is reached. The order from complex to simple is meta-protein, proteose, peptone, polypeptides, amino acids.

(a) **Meta-proteins.**—When a protein is treated either with acid or alkali at about 60° C. it goes into solution. This solution does not coagulate on boiling, but there is the formation of a precipitate when the solution is carefully neutralised. This precipitate is again soluble in excess either of acid or alkali. If the precipitate on neutralisation is filtered off and suspended in water coagulation will occur on boiling. The coagulum is no longer soluble, for example, in dilute acid. Acid meta-proteins differ from alkali meta-proteins in that they are precipitated from solution on saturation with sodium chloride. Both meta-proteins are precipitated on full saturation with magnesium sulphate and half saturation with

ammonium sulphate. Naturally they both give the ordinary colour reactions of protein.

**EXPERIMENT.** To some diluted egg-white add two or three drops of 10 per cent. HCl. Place in water bath at body temperature for five minutes. Acid meta-protein is formed.

Note.—(a) That no coagulum now appears on heating.

(b) It is precipitated by making the solution neutral or very faintly alkaline.

(c) It is salted out by *half* saturation with ammonium sulphate (like a globulin).

(d) If neutralised and suspended in water it is coagulated on boiling.

(b) **Proteoses.**—This group of substances is divided into two, (1) primary and (2) secondary proteoses, which are differentiated by precipitation with ammonium sulphate.

Primary proteose is a mixture of protoproteose and heteroproteose. Precipitation takes place on half saturation with ammonium sulphate. No coagulation takes place on boiling. A white precipitate forms on the addition of nitric acid; this precipitate disappears on heating, but reappears on cooling. They are almost completely precipitated by alcohol. Salts of the heavy metals like copper sulphate, mercuric chloride, etc., and tannic acid cause precipitation.

Secondary proteoses, sometimes called deuteroproteoses, are precipitated on full saturation with ammonium sulphate. They behave like primary proteoses except that the nitric acid and copper sulphate tests are negative.

(c) **Peptone.**—This substance is not precipitated by ammonium sulphate. It gives a very characteristic biuret reaction—a definite pink coloration. (If the test is done in the presence of ammonium sulphate a large excess of caustic soda must be added as the alkalinity of the solution must be due to sodium.) It is precipitated by tannic acid and lead acetate.

**EXPERIMENT.** Use the solution of Witte's peptone provided and perform the following tests:

(a) Biuret reaction is *pink*. (*Proteoses* and *Peptones*.)

(b) On faintly acidifying with acetic acid and boiling—*no coagulum*.

(c) Add a little  $\text{HNO}_3$ —a white ring. This dissolves on heating and reappears on cooling. Salicyl-sulphonic acid produces the same effect, but the reaction is more delicate.

(d) To the solution add an equal amount of  $(\text{NH}_4)_2\text{SO}_4$  (half saturate). A white precipitate of the *primary proteoses* is formed. Filter.

(e) Saturate the filtrate with crystals of  $(\text{NH}_4)_2\text{SO}_4$ . The *secondary proteoses* are precipitated. Filter.

(f) With the filtrate perform Biuret and xanthoproteic tests. As peptones are not precipitated by  $\text{HNO}_3$  the xanthoproteic test manifests itself by a yellow colour on heating the solution, turning

orange with ammonia. The positive results show the presence of *peptones*.

From these experiments we learn :

- (1) That proteoses and peptones give a pink biuret.
  - (2) That they are not coagulable by heat.
  - (3) That proteoses give a precipitate with  $\text{HNO}_3$  soluble on heating. Therefore, in the presence of other proteins, precipitated by  $\text{HNO}_3$ , such as albumin and globulin, they can be separated by warming the solution and filtering hot. The precipitates of albumins and globulins do not dissolve on warming.
  - (4) Primary proteoses are salted out by *half* saturation with ammonium sulphate.
  - (5) Secondary proteoses are salted out by *full* saturation with ammonium sulphate.
  - (6) All proteins but peptones are salted out by full saturation with ammonium sulphate.
- (The other products of protein hydrolysis are dealt with under Digestion, pp. 226 and 306.)

## CHAPTER IV

### CARBOHYDRATES

**Chemical Relationships.**—These are compounds of carbon, hydrogen, and oxygen, in which the latter two elements usually exist in the same proportion as in water. Their general formula is therefore  $\text{C}_m\text{H}_{2n}\text{O}_n$ .

Carbohydrates are found chiefly in vegetable tissues, but also occur in animal tissues. They form very important food-stuffs, for they are easily digested and assimilated, and moreover are much cheaper than proteins and fats. The simplest form of carbohydrate is called a *monosaccharide*, and all other carbohydrates can be broken down into two or more monosaccharide molecules by the chemical process of hydrolysis. When, by this process, two monosaccharide molecules are produced, the carbohydrate is called a *disaccharide*; when more than two are produced, the carbohydrate is called a *polysaccharide*. The monosaccharides and disaccharides being sweet to the taste are together spoken of as sugars.

#### I. Monosaccharides

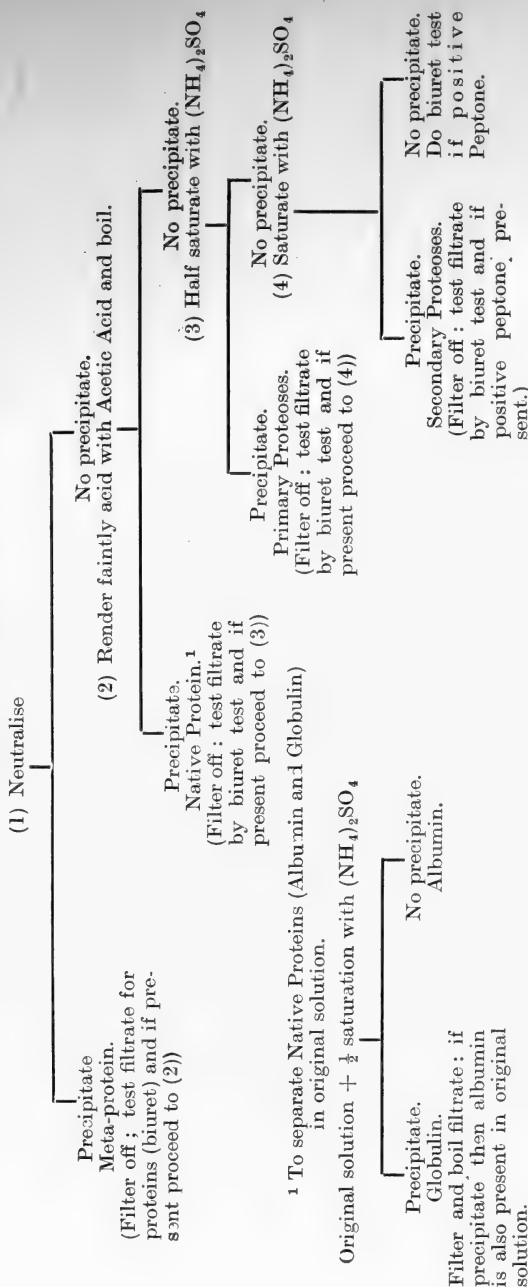
Chemically considered, monosaccharides are either aldehydes or ketones; the former are called aldoses, the latter ketoses. The sugars are classed according to the number of carbon atoms in the molecule, e.g. pentose  $\text{C}_5\text{H}_{10}\text{O}_5$ , hexose  $\text{C}_6\text{H}_{12}\text{O}_6$ .

**Aldoses.**—An aldehyde is the first oxidation product of a primary alcohol, and it contains the end group —CHO.

A *primary* alcohol is one in which the "OH" or "hydroxyl group" is

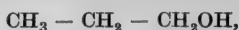
PROTEINS.

Take reaction of solution to litmus and do biuret test.  
If markedly acid or alkaline.



N.B.—Polysaccharides are also precipitated by neutral salts and may have to be tested for. Mucin and most nucleo-proteins behave like Primary Proteoses.

attached to the last carbon atom of the molecule—as in primary propyl alcohol,

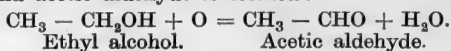


and it contains the end group  $-\text{CH}_2\text{OH}$ . If, on the other hand, the hydroxyl group be attached to a central carbon atom—as in secondary propyl alcohol,

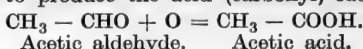


the alcohol is called *secondary*, and contains the group  $-\text{CHOH}$ .

If ethyl alcohol be heated with potassium bichromate and sulphuric acid, it is oxidised and acetic aldehyde is formed :



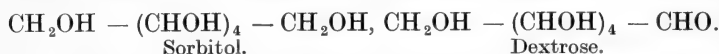
This group,  $-\text{CHO}$ , is, however, not a stable one, but very readily undergoes further oxidation to produce the acid (carboxyl) radicle  $-\text{COOH}$ ,



As a consequence of this tendency to absorb oxygen aldehydes are strong *reducing agents*, and it is this property which constitutes one of their most important group reactions, for the reaction is frequently accompanied by a visible change in the colour of the solution.

Their power of reducing cupric hydroxide, which is blue in colour, to cuprous oxide, which is red, and of reducing silver nitrate to metallic silver, is of especial value as a test. Similar reactions are obtained with certain bismuth and mercury salts. In order to obtain these reactions, it is necessary that the liquid be alkaline in reaction.

**Reactions of Monosaccharides depending on the fact that they are aldehydes.** *I. Their Reducing Power.*—Dextrose is the aldehyde corresponding to the hexatomic<sup>1</sup> alcohol, sorbitol.



It, therefore, manifests strong reducing powers on metallic oxides in alkaline solution.

**EXPERIMENT I.** Demonstrate the reducing power of a monosaccharide, such as dextrose on cupric salts in alkaline reaction.

*Trommer's Test.*—Place a few drops of a weak solution of copper sulphate in a test tube ; add about 5 c.c. of a 1 per cent. solution of dextrose, and then, drop by drop, a 20 per cent. solution of caustic soda until the precipitate of cupric hydroxide, which at first forms, becomes redissolved, and a clear blue solution is obtained. Boil. Reduction is effected, a red precipitate of cuprous oxide resulting.

Repeat experiment without the addition of dextrose. A black precipitate of cupric oxide is obtained on boiling with excess of caustic soda.

**EXPERIMENT II.** *Fehling's Test.*—This differs from Trommer's test in that tartrate of sodium and potassium (Rochelle salt) is added to the mixture of  $\text{CuSO}_4$  and  $\text{NaOH}$ .<sup>2</sup> Rochelle salt has the property of dissolving cupric hydroxide, forming a blue solution, which is unaltered on boiling, and is therefore of especial value

<sup>1</sup> A hexatomic alcohol is one which contains six OH groups. Glycerine is called tri-atomic, because it contains three such groups. Ethyl alcohol is mon-atomic, because it contains one.

<sup>2</sup> For the exact formula for Fehling's solution see p. 275.

when the solution to be tested contains only a small amount of dextrose or other reducing substance. Boil a few c.c. of Fehling's solution in a test tube. Add the dextrose solution drop by drop, with continued boiling, until reduction results, as evidenced by the diminution of the blue colour and the formation of an orange red precipitate.

EXPERIMENT III. *Benedict's Test*.—To 5 c.c. Benedict's (qualitative) reagent<sup>1</sup> add a few drops of dextrose solution and boil hard for several minutes. According to the amount of sugar present in the solution used the colour varies from greenish to red.

EXPERIMENT IV. *Nylander's Test*.—To about 5 c.c. of dextrose solution in a test tube add about 1 c.c. of Nylander's reagent (a solution containing 10 per cent. caustic soda, 4 per cent. Rochelle salt and 2 per cent. bismuth subnitrate). Boil for two minutes. A black precipitate of bismuth forms. Some substances (creatinine, uric acid) which reduce Fehling's solution do not give this test. As regards the sugars, however, where Fehling's test is positive this test will also be positive.

EXPERIMENT V. Boil glucose solution with Barfoed's<sup>2</sup> solution. Add two drops of the sugar solution to about 5 c.c. of Barfoed's solution in a test tube and boil for about half a minute. Remove from the flame, shake gently for 10–15 seconds and again boil. *The appearance of the red copper oxide indicates the presence of a monosaccharide.* (The reduction which takes place with Barfoed's solution is, as a rule, very slight.)

II. *Monosaccharides form compounds called Osazones, with Phenyl Hydrazine*.—The compounds are very useful in identifying the various forms of sugars, as each sugar forms a slightly different compound.

EXPERIMENT VI. The production of osazones. Add .25 gm. (enough to cover a sixpence) of phenyl-hydrazine hydrochloride and an equal bulk of sodium acetate crystals to about 10 c.c. of a 1 per cent. solution of dextrose. Warm gently till everything is dissolved, and then place for half an hour in a boiling water bath. Allow to cool, when a yellow precipitate of glucosazone will separate out. Examine this under the microscope, and notice that the precipitate is composed of branching needle-shaped crystals arranged in rosettes or sheaves (Fig. 173).

The chemical reaction takes place in two stages, the intermediate body being called a hydrazone.

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<sup>1</sup> Benedict's (qualitative) reagent. Anhydrous sodium carbonate 100 grms. and sodium citrate 175 grms. are dissolved in about 500 c.c. water; to this mixture is added 17.3 grms. crystalline copper sulphate dissolved in 100 c.c. water, gradually, and with frequent shaking. Make up to 1 litre. The advantage of this solution is that it does not deteriorate on keeping, it does not destroy traces of sugar, and is not reduced by chloroform.

<sup>2</sup> Barfoed solution: 50 grms. cupric acetate and 50 grms. sodium acetate are dissolved in water, 5 c.c. glacial acetic acid added and the solution made up to 1 litre with water.

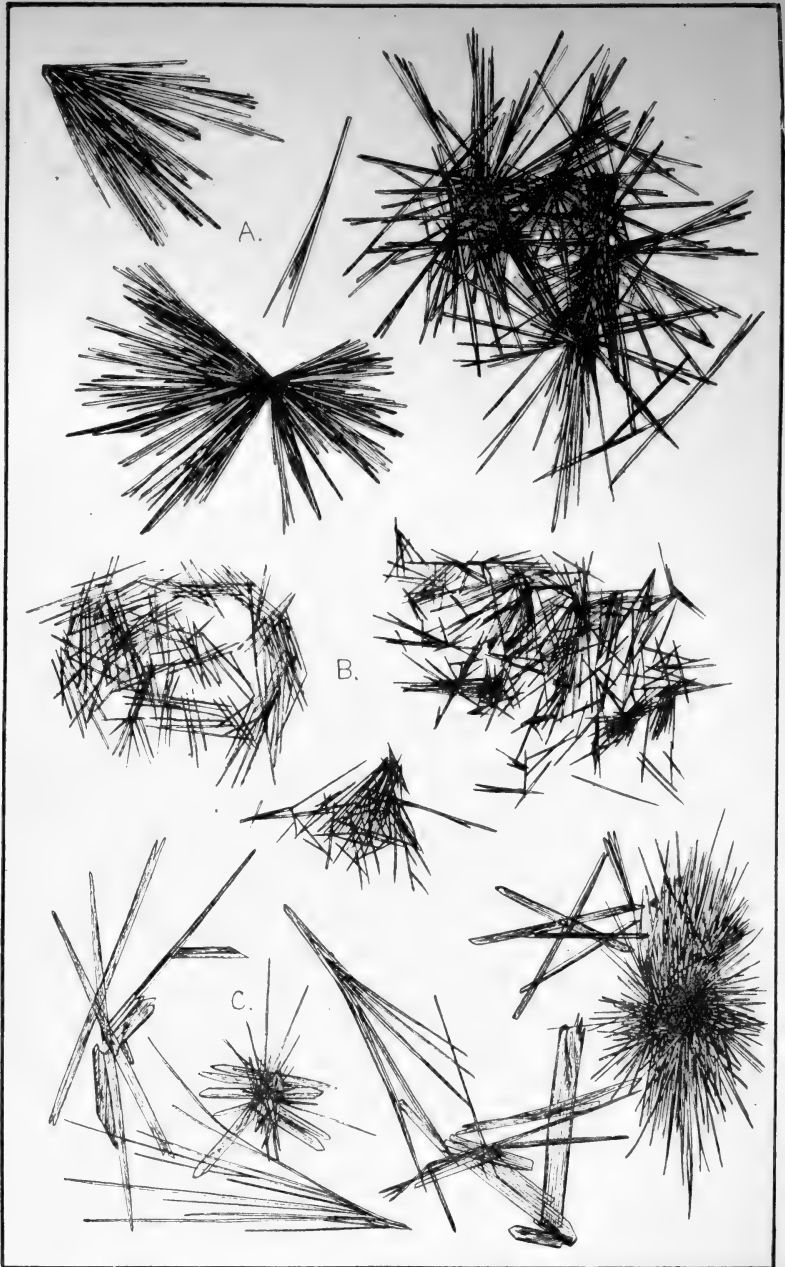


FIG. 173.—Osazone crystals.  $\times 400$ .  
A, Phenyl-glucosazone ; B, Phenyl-maltosazone ; C, Phenyl-lactosazone.



The excess of sodium acetate in the above mixture reacts with the phenyl-hydrazine hydrochloride so as to form the acetate.

When it is desired to obtain osazones from dilute sugar solutions, a more certain way to proceed is as follows:—Mix two drops of phenyl-hydrazine (fluid) with ten drops glacial acetic acid and add to this 5 c.c. of the sugar solution, shake, and place the test tube for one hour in the boiling water bath. After cooling examine under the microscope for the crystals. With stronger sugar solutions this method yields crystals after a few minutes' heating.

The advantage of the phenyl-hydrazine hydrochloride is that it does not readily decompose on keeping, whereas the free base does.

The osazone crystals are valuable for distinguishing between the different sugars. Besides microscopical examination, a determination of the melting point is often of value.<sup>1</sup> For this purpose the crystals of osazone are collected on a filter paper, washed with water acidulated with acetic acid, recrystallised from water, alcohol or acetic acid, and dried by placing in a desiccator over  $H_2SO_4$ . They are then placed in a narrow glass tube closed at one end and tied on to the bulb of a thermometer by a fine platinum wire. The thermometer is suspended in a long-necked flask (about 50 c.c. capacity) in which is concentrated  $H_2SO_4$  (almost saturated with  $K_2SO_4$  to prevent fuming) and the temperature gradually raised by heating the flask over wire gauze. The bulb of the thermometer should dip into the sulphuric acid. The exact temperature at which the crystals begin to melt and the temperature of complete fusion are noted. For accurate work, a correction is necessary because the mercury thread is cooler than the bulb of the thermometer.<sup>2</sup>

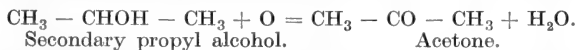
The following are the melting points of some of the most important osazones:

Dextrosazone, <sup>3</sup>	. . .	204–205° C.
Lactosazone	. . .	200° C. (Begins to melt at this temp.).
Maltosazone	. . .	206° C.

If the crystals are pure, melting occurs at once, but if they are impure there may be a considerable difference in temperature between the points of commencing and complete fusion.

**Ketoses.**—As mentioned above, some carbohydrates belong to the group of substances called ketones.

A ketone is the oxidation product of a secondary alcohol and it contains the group —CO— which is situated somewhere in the chain between other groups and not at the end of it as in the case of the —CHO group of the aldehydes. The simplest ketone is acetone  $CH_3 - CO - CH_3$  which may be obtained by oxidation of secondary propyl alcohol,



Ketones form compounds with phenyl hydrazine, but only some of them reduce metallic oxides in alkaline solution. Those ketones which belong to the carbohydrates manifest this reducing power. The only well-known ketose is lævulose.

There are several reactions characteristic of ketoses, of these the following is important.

<sup>1</sup> Too much reliance must not be placed on a determination of the melting points of osazones in identifying unknown sugars, for they vary with the rate of heating and with the method of purification of the osazone.

<sup>2</sup> To make the above correction, a second thermometer must be suspended in the flask with its bulb opposite the middle of the column of mercury of the main thermometer, the formula for correction is then  $L(T - t)$  (0.000154) where  $L$  = the height of the mercury column of the main thermometer above the sulphuric acid measured in degrees;  $T$  = the reading of the main thermometer;  $t$ , the reading of the air thermometer. This correction must be added to the reading  $T$  of the main thermometer.

<sup>3</sup> Lævulose forms the same osazone as dextrose.

**EXPERIMENT. Seliwanoff's Test.**—Mix a few cubic centimeters of a solution of lævulose with half its volume of concentrated HCl. Add a few crystals of resorcin and heat the mixture. A deep red colour develops and later a brown precipitate. The colour can be extracted by shaking with amyl alcohol. The characteristic red colour should appear before the solution boils.

Repeat this experiment with pure dextrose solution instead of lævulose. A slight red colour develops but no precipitate.

## CHAPTER V

### CARBOHYDRATES—*Continued*

There are, however, other reactions of carbohydrates which do not depend on their being aldehydes or ketones. The most important of these are :

*I. Molisch Test.*—This is an extremely sensitive test, being especially suitable for the detection of minute traces of carbohydrate. For example, most proteins (e.g. egg albumin) give it, on account of the carbohydrate groups which they contain.

**EXPERIMENT I.** To about 2 c.c. of a very dilute sugar solution, or of a strong solution of egg albumin, add a drop of a saturated alcoholic solution of  $\alpha$ -naphthol. Then carefully pour about an equal volume of pure concentrated  $H_2SO_4$  down the wall of the test tube so that it forms a layer at the bottom. On standing a minute or so a deep violet ring forms at the line of contact of the two fluids. The greenish colour which also develops is due to the reagents and is no part of the test.

*II. Fermentation with Yeast.*—By allowing yeast to grow on a solution of dextrose, the latter is split up into alcohol and carbon dioxide,



All carbohydrates do not give this reaction, so that it is of value as a distinguishing test for the presence of dextrose in the urine. Commercially it is the agency employed in the preparation of alcoholic beverages.

To ascertain whether the addition of yeast to a solution produces fermentation, the process should be allowed to proceed in an inverted tube over mercury, or in a Southall's ureometer so that any carbon dioxide gas which develops may be collected, and if necessary tested.

**EXPERIMENT II.** Shake up a 1 per cent. solution of dextrose, which has been previously boiled to expel the air and then cooled, with a piece of yeast the size of a split pea. Pour the opalescent solution thus obtained into a Southall's ureometer so that it completely fills the vertical tube. Now place the tube aside in a warm place for some time, when it will be found that a certain amount of gas has collected at the top of the tube. This gas is  $CO_2$  as can be shown by adding some NaOH to the tube by means of a pipette and shaking; the gas disappears. As a control, a tube filled with water and yeast should also be incubated. This should yield no gas.

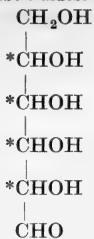
**EXPERIMENT.** Repeat the above experiment with similar solutions of maltose, lactose and cane sugar, and note that, after 24 hours, lactose has not undergone any fermentation, whereas it is marked in the case of maltose; cane sugar may also show a certain amount of fermentation. Yeast contains

an invertase (maltase) which readily hydrolyses maltose into dextrose, on which the zymase of the yeast then acts, forming alcohol and carbon dioxide. Another invertase in the yeast acts on cane sugar. These invertases have no action on lactose.

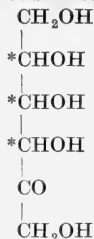
**III. Rotation of Polarised Light.**—All simple carbohydrates rotate the plane of polarisation of polarised light to the right except lævulose, which rotates to the left.

This effect is due to the presence in the molecule of asymmetrical carbon atoms.

6 carbon aldose (hexose).



6 carbon ketose.



\* Denotes an asymmetrical carbon atom.

Examination of the above formulæ shows that the aldoses contain four asymmetrical carbon atoms, whilst the corresponding ketoses contain only three. The different arrangements in space of the hexose carbon atoms allow of the existence of sixteen different hexoses, of which twelve have been identified. Only two, however, are of physiological importance, dextrose and galactose. The different spatial arrangement of the atoms in the molecule accounts for the difference in rotatory powers shown by these aldoses and also for slight differences in chemical properties, such as crystalline form and melting point of the osazones.

**Polarisation of Light.**—When two slices of tourmaline, a semi-transparent mineral, are cut parallel to the axis of the crystal and laid over one another, it will be noticed that the amount of light which passes through the combination varies according to the relative positions of the two slices to one another. If the slices be at right angles to one another no light passes through, and in intermediate positions only a certain amount, so that an opaque combination is obtained. A ray of ordinary light contains vibrations in all planes passing through the ray; but when the light passes through a tourmaline plate it vibrates in one plane only. Ordinary light may, therefore, be likened to a wheel, the axle representing the ray of light and the spokes the planes along which it vibrates. On passing through the tourmaline plate, however, the light is capable of vibrating in one plane only, which would correspond, in our example, to two opposite spokes. The light which vibrates in one plane is called plane-polarised light, and cannot be distinguished by the naked eye from ordinary light. By placing a second, similarly cut, tourmaline plate in its course, however, it can be detected, for it will pass through this only if its axis corresponds to the axis of the first plate. The first plate is called the *polariser* and the second plate the *analyser*. The mechanism of this action of the analyser and polariser can be easily illustrated by a piece of string stretched between two posts; it can vibrate in all planes. If a comb be placed in the course of the string the vibrations can only take place along one plane corresponding to the direction of the teeth of the comb. This comb represents the polariser. If now, a second comb be placed along the string it will permit the vibration of the string or stop it, according to the position of its teeth; if these be in the same direction as those of the first comb the string will go on vibrating, but if they be placed at right angles the string will cease to vibrate. Polarisation of light by tourmaline illustrates the principle of the polarimeter,

but in this instrument itself it is found more convenient to use a polariser and analyser made of a *Nicol's prism*. A Nicol's prism consists of a crystal of Iceland spar. Such a crystal has the power of splitting light into two rays, one of which, the *ordinary ray*, passes through it as it would through glass, and the other one, the *extraordinary ray*, is more refracted. Consequently, on looking at a dot on a sheet of paper through a piece of Iceland spar laid flat on the paper, a double image of the dot is obtained, and if the crystal be rotated, one of the dots—the extraordinary ray—will be seen to move round the other—the ordinary ray—which remains stationary. Now both these rays are polarised, but in different planes. If the crystal be cut across along a diagonal line and the two surfaces re-cemented by means of Canada balsam, the ordinary ray, when it meets the balsam, will be totally reflected and pass out at the side of the crystal, whereas the extraordinary ray will be transmitted through the balsam, and will finally emerge at the end of the prism,

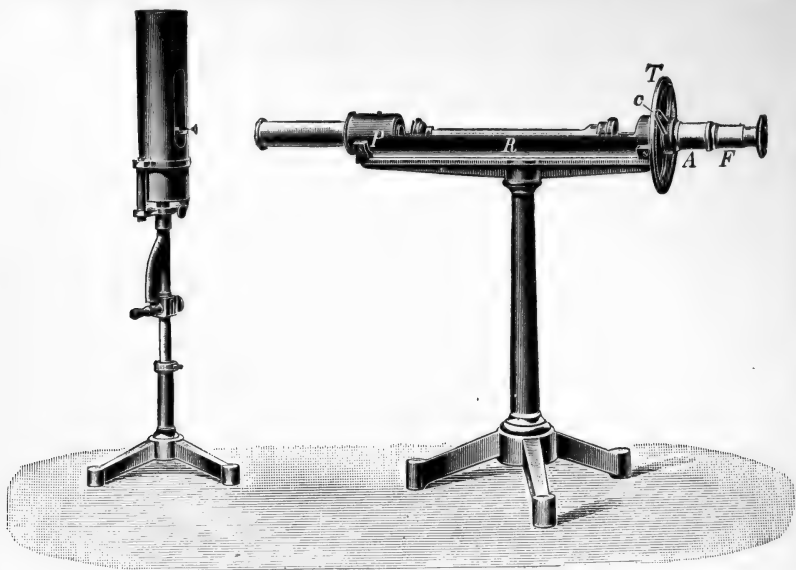


FIG. 174.—Polarimeter of Mischerlich with Laurent's polariser.

*P*, polariser and device for obtaining half shadow; *R*, fluid container; *T*, scale with vernier *c* attached to pointer; *A*, compensator and analyser; *F*, lens.

parallel to its original direction; but, of course, plane polarised. To detect the polarisation a similarly constructed prism, or analyser, must be used.

Certain other bodies, e.g. a quartz plate, a solution of sugar or albumin, have the power of *rotating the plane of polarised light*. Thus, supposing that the plane polarised light vibrates along a vertical plane, one of these bodies will cause it to vibrate in an oblique plane. If the analyser be so placed that none of the plane polarised light can pass through it (i.e. the field is black), and if a piece of quartz be inserted between the polariser and analyser, it will be found that now a certain amount of light passes through the analyser (i.e. the field becomes opaque), and, in order to obtain darkness again, it is necessary to rotate the analyser in the direction of the hands of a watch, as seen by the observer. Consequently, rotation has taken place to the right, i.e. dextro rotation is said to have occurred. If a solution of albumin or levulose be employed the rotation of the analyser must be to the left, i.e. against the hands of the watch. When the plane of white light passes through the quartz

plate, however, the various colours of the spectrum are rotated to a different degree, so that, instead of having a mere opacity (as is the case with intermediate positions of two "tourmaline" plates), different colours are obtained according to the amount of rotation. There are also samples of quartz which rotate the plane of light to the left.

Dextrose and a quartz plate produce the same amount of rotation, and therefore it is possible to determine the rotatory power of a solution of the former by compensating its rotation by means of a quartz plate of known rotatory power.

We are now in a position to understand the construction of a **polarimeter** or **saccharimeter**. It consists of the following parts:

(1) A Nicol's prism, called the *polariser*. This polarises light in a vertical plane.

(2) A biquartz, or other device for rotating, in opposite directions, the two halves of a polarised beam. A biquartz consists of a disc of quartz made of two semicircular halves of equal thickness, but of opposite rotatory powers. Each half is of such a thickness that it rotates the plane polarised light to  $90^\circ$  in opposite directions so that, on emerging from the disc, the plane of light is now horizontal. Instead of a biquartz many instruments contain a semicircular plate of quartz.

(3) A tubular **liquid holder** to hold 10 c.c. of the liquid to be examined. If the length of this tube be 188.6 mm. the amount of rotation in angular degrees will correspond to percentage of dextrose in the fluid (e.g. urine) examined.

(4) A **Compensator**.—This shows how much rotation has been produced by the solution. It is connected with a scale representing angular degrees, and the pointer carries a vernier, so that tenths of a degree can be read off. In some instances the compensator consists of two wedge-shaped pieces of quartz, so arranged on one another that the total thickness of quartz interposed in the path of the polarised beam can be varied by means of a screw. In other instruments the quartz plates are dispensed with, the amount of rotation being measured by rotating the next part of the instrument, namely, the

(5) **Analyser**, so as to obtain uniformity of tint in the two halves of field.

(6) A **Lens**.

When the tube (3) is filled with water or an optically inactive fluid, and the compensator or analyser rotated until a violet colour of uniform tint fills the field, the indicator will be seen to stand at zero (if not so, the error must be noted). If now, an optically active fluid be placed in the tube the two halves of the field will become of different tints, i.e. rotation of the plane of polarised light has occurred. In order to measure the amount of this rotation, we must move the screw or pointer connected with the compensator or analyser until the uniform tint is again obtained.<sup>1</sup> The amount of "compensation" necessary is read off on the scale and, if the holder be not 188.6 mm. long, the necessary calculation is made in order to ascertain the strength of the solution (for formula see below).

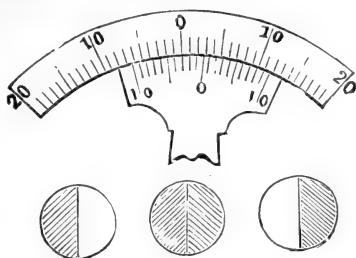


FIG. 175.—Diagram of scale and field of vision of polarimeter.

Above is represented the scale for measuring the compensation necessary. In the position represented in the diagram the reading is 2.7 dextro rotation. The lower part of the diagram shows the three appearances of the field of the polarimeter, the central one representing the appearance at zero, i.e. when there is no rotation.

In other instruments the quartz plates are dispensed with, the amount of rotation being measured by rotating the next part of the instrument, namely, the

<sup>1</sup> In the best modern polarimeters the field is divided into three; when at zero these are of the same tint, otherwise the central band takes a different colour.

To estimate the percentage of sugar in urine the chief precautions are, (1) to see that it is *perfectly clear*, and (2) to see that it contains no protein.

In order to obtain a specific or comparative number (i.e. a result always obtained under the same conditions) it is necessary to adopt a standard. This consists of the rotation, in degrees of a circle, produced by 1 gr. of the substance dissolved in 1 c.c. of fluid and contained in a tube 1 decm. long. This is called the *specific rotatory power* and is represented by  $(\alpha)D$ .<sup>1</sup> It is determined by the following formula :

$$(\alpha)D = + \frac{a}{p \times l}$$

where  $a$  = the observed rotation,

$l$  = the length, in decimeters, of the tube in which the solution is placed,

$p$  = the weight, in grammes, of the substance contained in 1 c.c. solvent.

The rotation produced by a substance depends upon its concentration in a solution ; if, therefore, the index  $(\alpha)D$  of any substance be known, and its rotation be ascertained, its percentage  $P$  in any fluid can be ascertained by the formula :

$$P = \frac{100a}{s l}$$

where  $s = (\alpha)D$ .

For rapidly and accurately determining the percentage of sugar in any fluid (e.g. urine) the polarimeter—and especially that form of it in which the scale reads percentages of sugar—is a very valuable instrument. It is much used for this purpose in the continental clinics.

The Specific Rotatory Power<sup>2</sup> of certain of the sugars in about 10 per cent. solution is as follows :

<i>Monosaccharides</i> :	Dextrose :	+ 52.7°.
	Galactose :	+ 83°.
	Lævulose :	- 93°.

*Disaccharides*.—The  $(\alpha)D$  of these carbohydrates changes when they are hydrolysed.

Cane sugar : + 66.5°—after hydrolysis becomes laevorotatory.

Maltose : + 138°—after hydrolysis becomes much less.

Lactose : + 52.5°—after hydrolysis becomes slightly more.

*IV. Moore's Test*.—When heated with caustic soda a dark substance called caramel is produced. This is also produced when sugar is burnt. Caramel contains several chemical bodies, the most important of which is an acid called levulinic acid.

Quantitative methods for the estimation of sugar will be found on pp. 275 and 292.

**The Chief Monosaccharides** are dextrose, lævulose and galactose.

**Dextrose**, grape sugar or glucose ( $C_6H_{12}O_6$ ), is found in many fruits, and is an important food-stuff. In the healthy animal body it occurs in the blood and muscles. In normal human blood the amount of glucose is usually from 0.1 to 0.15 per cent.

It is soluble in water and in alcohol. It has only a slightly sweet taste. It rotates polarised light to the right ( $(\alpha)D = + 52.7$ ).

<sup>1</sup> The "D" indicates that sodium light is used.

<sup>2</sup> The rotatory power of a solution of a sugar is frequently different when the solution is freshly made from what it becomes on standing. This phenomenon is called mutarotation. The figures given are all for solutions which have been kept long enough to be in equilibrium. Temperature also affects the rotatory power of a solution, particularly in the case of lævulose and invert sugar.

Glucose readily combines with alcohols, acids, phenols, etc., to form *glucosides*. These are resolved into their constituent groups by hydrolysis with acid.

**Lævulose** ( $C_6H_{12}O_6$ ) is found along with dextrose in fruits and honey and results from the hydrolysis of cane sugar (see Disaccharides). It is very rarely found in animal tissues. It is crystallisable with great difficulty, being usually obtained as a putty-like mass. It is lævo-rotatory ( $(\alpha)D = -93^\circ$ ).

**Galactose** ( $C_6H_{12}O_6$ ) is a dextro-rotatory sugar produced, along with dextrose, by hydrolysing lactose (see Disaccharides). Certain lipid substances in brain tissue, yield galactose on hydrolysis. It differs but slightly from dextrose in its reactions. Its presence can be detected by the fact that when oxidised, as by boiling with nitric acid, it yields mucic acid which forms characteristic crystals.

**EXPERIMENT.** Test for galactose. Add 3 c.c. pure  $HNO_3$ (con.) to 10 c.c. of a strong solution of lactose in a small evaporating dish. Boil gently over a free flame for three minutes, and then lower the flame and allow to evaporate till the volume is reduced to about 3 c.c. Transfer to a test tube, cool under the tap, add 2 c.c. water, and allow to stand. Crystals of mucic acid separate out.

## II. Disaccharides

Chemically, each molecule of a disaccharide consists of two molecules of a monosaccharide less one molecule of water,



Their structure can be demonstrated by hydrolysing them, i.e. by causing them to take up a molecule of water, in consequence of which they split up into two monosaccharides. In disaccharides the two monosaccharide molecules are linked together in the same manner as glucose and the other constituent radicle in glucosides.

The chief means of hydrolysing include boiling with *dilute* acid and the action of certain ferments called invertases, which are contained in the succus entericus and in the protoplasm of many cells such as the yeast plant (see p. 210).

The members of this class are cane sugar, maltose and lactose, and of these cane sugar does not reduce metallic oxides in alkaline solution, nor does it form an osazone, whereas lactose and maltose give both these reactions. With yeast maltose and cane sugar are first hydrolysed, and the monosaccharides thus produced then undergo alcoholic fermentation.

**Cane Sugar or Sucrose** ( $C_{12}H_{22}O_{11}$ ) is the common sugar obtained from sugar cane, beetroot, etc. It is very soluble in water and has a sweet taste. It does not reduce metallic oxides in alkaline solution.

**EXPERIMENT I.** Perform Trommer's test with some cane sugar solution. Notice that, although no reduction occurs, the cane sugar, like other sugars, is capable of holding the cupric hydroxide in solution, so that a clear blue colour is produced.

By hydrolysis, reducing sugars (dextrose and lævulose) are developed.

**EXPERIMENT II.** Boil some cane-sugar solution with a few drops of 25 per cent. sulphuric acid. Now neutralise the acid and apply Trommer's or Fehling's test and note that reduction occurs. The monosaccharides formed are dextrose and lævulose, the mixture being called invert sugar.

It is often better to employ an organic acid such as citric acid to produce the hydrolysis, because the organic acid does not hydrolyse starch or glycogen, whereas mineral acids do.

**EXPERIMENT III.** Apply Seliwanoff's test for ketose to a solution of cane sugar. The reaction is as marked as for lævulose, owing to hydrolysis of the cane sugar by the hydrochloric acid employed.

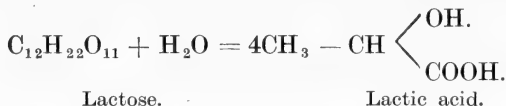
**EXPERIMENT IV.** Heat some cane sugar solution with strong hydrochloric acid. Note the reddish colour developed. This reaction is given by other sugars, but not so readily.

A solution of cane sugar is dextro-rotatory ( $(\alpha)D = +66.5$ ), but after hydrolysis it is lævo-rotatory, the lævo-rotatory power of the lævulose being stronger than the dextro-rotatory power of the dextrose formed. On this account the process of hydrolysis is sometimes called inversion, and the hydrolysing ferments in the succus entericus, etc., are often called invertases.

**EXPERIMENT.** Examine a 10 per cent. solution of cane sugar with the polariscope. Note the rotation and calculate  $(\alpha)D$ . Place exactly 50 c.c. of a 20 per cent. solution of cane sugar in a 100 c.c. measuring flask; add 1 gr. citric acid and boil over wire gauze for five minutes. Cool, neutralise with NaOH solution, and fill with distilled water to the 100 c.c. mark. Examine this solution with the polariscope and calculate  $(\alpha)D$ .

**Lactose** ( $C_{12}H_{22}O_{11}$ ) is the sugar found in milk, and it has been detected in the urine of nursing mothers.

It is not very soluble in water, and is quite insoluble in alcohol and ether. It has only a slightly sweet taste. It does not ferment with yeast in twenty-four hours, but it undergoes a special fermentation with the bacillus acidi lactici which develops in sour milk. This fermentation results in the production of lactic acid.



By hydrolysis it yields dextrose and galactose. It reduces metallic oxides in alkaline solution. It is dextro-rotatory ( $(\alpha)D = +52.5$ ). By oxidation with nitric acid it yields mucic acid.

**Maltose** ( $C_{12}H_{22}O_{11}$ ) is important physiologically because it is the sugar produced from starch by the diastases present in the digestive juices and tissues. Maltose is therefore mainly an intermediate substance in the animal body.

Maltose is also produced by the action of malt diastase, which is obtained by moistening barley and allowing it to germinate in heaps at a constant temperature. The diastase acts on the starch of the grain and produces maltose. The product when dried is called *malt*. When malt is dissolved in water, and the yeast plant allowed to grow on the solution, malted liquors,



such as beer and ale, are obtained. In this process the maltose is first of all inverted into two molecules of dextrose by the invertase contained in the yeast, and the dextrose then undergoes alcoholic fermentation.

It reduces metallic oxides in alkaline solution, but is feebler in this regard than dextrose. It rotates the plane of polarised light more strongly than dextrose ( $(\alpha)D = +138^\circ$ ). After hydrolysis, therefore, the reducing power shows an increase and the rotatory power a decrease.

**EXPERIMENT.** Boil lactose or maltose solution with Barfoed's reagent. There is no reduction. This reagent is not reduced by disaccharides.

**Isomaltose.**—This sugar is closely related to maltose, differing from it in the fact that its osazone melts at a much lower temperature,  $158^\circ\text{C}$ . It has been prepared by pure chemical synthesis—e.g. the condensation of dextrose by strong acids. It is of special interest because it is probably the sugar produced as a result of the reversible action of maltase.

## CHAPTER VI

### CARBOHYDRATES—*Continued*

#### III. Polysaccharides.

A polysaccharide is the condensation product of more than two monosaccharide molecules, and has accordingly the general formula  $(\text{C}_6\text{H}_{10}\text{O}_5)_n$ , where  $n$  stands for a variable number.<sup>1</sup> Polysaccharides can be hydrolysed, in which process they yield, first of all, polysaccharides (dextrines) of lower molecular weight (i.e. with  $n$  of less value), then disaccharides and, finally, monosaccharides.

Thus, when acted on by diastatic ferments, dextrines (polysaccharides of lower molecular weight) and maltose (disaccharide) are formed. When boiled with acid, on the other hand, the hydrolytic cleavage goes further, and, although dextrine and maltose occur as intermediate products, the final product is monosaccharide.

The most important members of this group are the starches, the dextrines, glycogen, the celluloses, and the gums. They are very widely distributed in vegetables, and constitute a most important class of food-stuffs.

**General Characters.**—They do not form crystals, nor, with few exceptions, are they soluble in cold water. Few possess any sweet taste. As a rule they do not diffuse through parchment and are therefore colloids. Their solutions are optically active. They do not reduce metallic oxides in alkaline solution, they do not form osazones and they cannot be fermented with yeast. Like other colloids, they are precipitated when their solutions are saturated

<sup>1</sup> It is impossible to give a definite value to  $n$  because the molecular weight is unknown. The symbol  $n$  signifies that the formula within the brackets is to be multiplied an indefinite number of times.

with certain neutral salts, such as ammonium sulphate. They may be sub-divided into three sub-groups, the starches, the dextrines and the celluloses.

**1. The Starches.**—These include ordinary starch and glycogen ( $C_6H_{10}O_5$ )<sub>n</sub>. Starch is the most widely distributed carbohydrate in the vegetable kingdom, for it is in this form that plants store up their excess of carbohydrate. Animals store their excess of carbohydrate partly as glycogen, but mainly as fat. If the amount of dextrose produced in the leaves be in excess of the immediate needs of the plant, it is stored up as starch.

The exact shape of starch grains varies according to the plant from which they are obtained. In this connection they may be divided into two groups: (1) a group in which the contour of the grains is even, such as wheat, barley, arrowroot, potato; (2) a group in which the contour is marked by facets, either completely, as in oats and rice, or only partially, as in tapioca and sago.

**EXPERIMENT I.** Examine some wheat flour, a scraping of potato, and some ground rice under the microscope. To do this, mix the flour, etc., with a drop of water on a slide, and examine under a cover slip.

Starch, like most other polysaccharides, is insoluble in cold water, but it swells up in hot water, an opalescent mixture being formed.

**EXPERIMENT II.** Place some powdered starch in a test tube, and half fill up with cold water—no solution occurs—now boil, when an opalescent mixture will be produced, and, if of sufficient concentration, this will gelatinise on cooling. Try Trommer's test—no reduction occurs.

The standard test for starch is with iodine solution.

**EXPERIMENT III.** To an opalescent cold solution of starch add a drop or two of a very dilute solution of iodine in potassium iodide; a blue colour results, which disappears on gradual heating and returns again on cooling. Excessive heat must be avoided, since the iodine is volatile.

Starch granules also give this reaction under the microscope. The cut surface of a potato gives it.

Hydrolysis can be effected by boiling with a weak acid or by the action of ferments such as ptyalin, amylopsin, and malt diastase.

**EXPERIMENT IV.** Place some starch solution in a test tube, add to it a few drops of 25 per cent. sulphuric acid and boil for five minutes. Neutralise and apply the iodine test and note that, instead of a blue, a reddish brown colour is produced (due to dextrine). Apply Trommer's or Fehling's test, and note that reduction occurs.

**EXPERIMENT V.** Place some of the starch solution in the mouth, and after a few minutes transfer it again to the test tube; now apply Trommer's or Fehling's test—reduction occurs.

Try the same experiment with some unboiled starch, and note that there is no reduction (i.e. the resistant external layers have not been hydrolysed).

**Glycogen** ( $C_6H_{10}O_5$ )<sub>n</sub>.—Just as plants store up excess of carbohydrate in the form of starch, so do animals store it partly in the form of glycogen. The chief seats of this storage are the liver and muscles. Glycogen forms an amorphous white powder. It is soluble in water and the solution is opalescent. Solutions of glycogen are dextro-rotatory and are precipitated by basic lead acetate solution.

**EXPERIMENT VI.** A simple method for the preparation of glycogen is that introduced by Fränkel. It consists in grinding up fresh liver or common shell-fish, mussel or oyster in a mortar with about three times its volume of a 3 per cent. solution of tri-chloroacetic acid. This reagent coagulates the proteins. The glycogen is contained in the extract, and can be precipitated by alcohol.<sup>1</sup> After collecting on a filter dissolve some of the glycogen in water and notice that the solution is opalescent. Add to this a drop or two of iodine solution: a port-wine colour results, which disappears on heating, and returns on cooling.

**EXPERIMENT VII.** Place 5 c.c. of glycogen solution in a test tube and add ordinary alcohol carefully until a precipitate forms. Note approximately how much alcohol requires to be added to obtain this (about 55 per cent.).

**EXPERIMENT VIII.** Try Trommer's test with the glycogen solution; no reduction occurs, but the  $Cu(OH)_2$  is held in solution.

**EXPERIMENT IX.** To some of the glycogen solution add a few drops of 25 per cent.  $H_2SO_4$  and boil for about ten minutes; dextrose is produced, as can be shown by applying one of the reduction tests.

**EXPERIMENT X.** Mix some glycogen solution with saliva and place the test tube in water at body temperature. After about ten minutes apply one of the reduction tests. It will be found that a reducing sugar has been produced.

**The Dextrines** ( $C_6H_{10}O_5$ )<sub>n</sub>.—During the hydrolysis of starch and glycogen dextrines are formed as intermediate products.

There are several varieties of dextrine, amylo-dextrine, erythro-dextrine and achroödextrine, which differ from one another in molecular weight, colour reaction with iodine, etc.

Dextrine is an amorphous powder, soluble in cold water forming a clear solution and is not precipitated by basic lead acetate.

**EXPERIMENT XI.** Add some iodine solution: a brownish red colour, like that obtained with glycogen, results if erythro-dextrine be present. The colour disappears on heating and reappears on cooling. The bluish violet tint frequently obtained is due to the presence of starch.

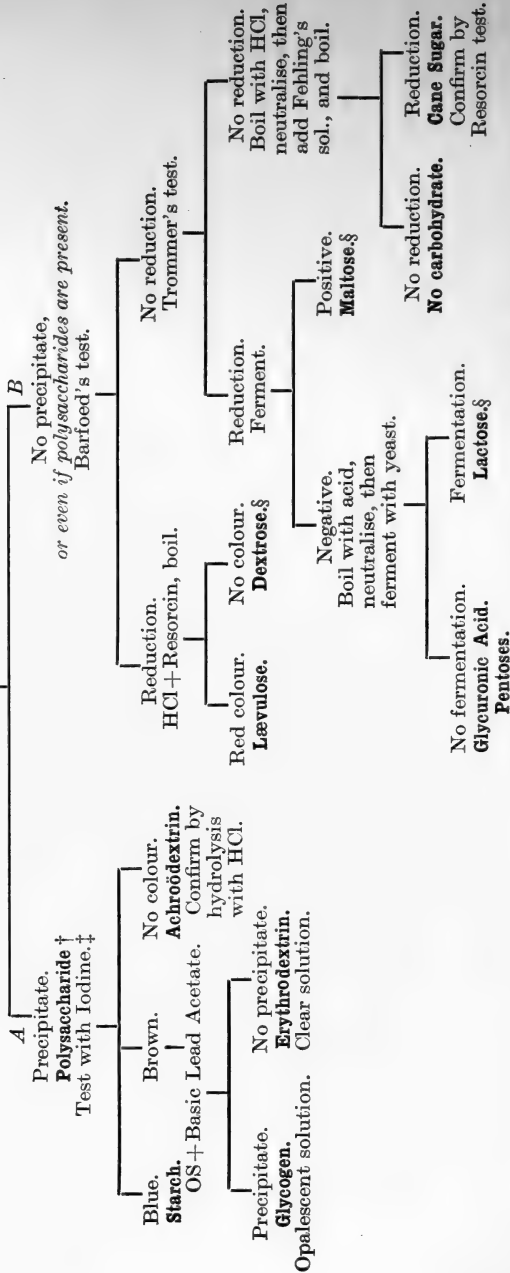
**EXPERIMENT XII.** Test reducing power of a solution of dextrine before and after hydrolysis with acid.

**Pentoses.**—Besides the hexoses, animal tissues also contain small amounts of pentoses, that is, sugars containing five carbon atoms,  $C_5H_{10}O_5$ . Being aldehydic in nature, they possess reducing powers and form osazone crystals. They do not ferment with pure yeast, but they all rotate the plane of polarised light. In the animal tissues pentoses do not exist in a free state, being, as far as is known, bound to guanylic acid. They are very plentiful in plants, where they exist as polysaccharides called *pentosans*. Thus, in gum arabic there is a pentosan which yields *l*-arabinose when hydrolysed by heating with mineral acid, and in wood or bran another pentosane yields *l*-xylose on

<sup>1</sup> Where not otherwise specified in these experiments, alcohol refers to the commercial product containing from 92 to 96 per cent. pure alcohol.

**CARBOHYDRATES.**

ALCOHOL.\*



\* Alcohol also precipitates proteins from solution.

† Solution must be cool and not alkaline.

‡ If these are present, test also by Column B for sugars.

§ Confirm by means of Phenylhydrazine.

similar treatment, which is the variety of pentose present in the nucleic acid of animal cells. Pentose sometimes occurs in the urine—the condition being called pentosuria—the variety being racemic arabinose (inactive optically). From what source this is derived is difficult to determine, for it is independent of the pentoses in the food, and its structure is different from that found present in the tissues (see p. 325).

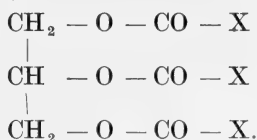
## CHAPTER VII

### FATS

Fats may generally be recognised by their various physical properties. They are insoluble in water, many are soluble with difficulty in cold alcohol, they are freely soluble in ether, benzene, chloroform, petrol, carbon disulphide, carbon tetrachloride, etc. Liquid fats and fats of low melting-point give persistent "greasy" (translucent) marks on paper. (The so-called "essential oils" also give such marks but they do not persist—they evaporate.) Two or three reagents such as Osmic acid and Sudan III give characteristic colour reactions. These substances are used as stains in Histology for the detection of fatty matter in sections.

#### Fats and Fatty Acids

**Neutral Fats** are the ethereal salts of the fatty acids with the tri-atomic alcohol glycerol, and have therefore the general formula:—



They are named according to the fatty acid they contain, thus: stearin, olein. *The fatty acids* are monobasic organic acids, containing one carboxylic group (COOH) attached to a hydrocarbon radicle. They belong to two classes, the saturated and the unsaturated. The saturated acids have the general formula  $\text{C}_n\text{H}_{2n+1} \cdot \text{COOH}$ . Those commonly occurring in fats are stearic acid, in which  $n = 17$ , and palmitic acid, in which  $n = 15$ . Thus the formula for stearic acid is  $\text{CH}_3 \cdot (\text{CH}_2)_{16} \cdot \text{COOH}$ .

The unsaturated acids contain relatively less hydrogen in the hydrocarbon chain attached to the carboxylic group. This is due to the fact that there are one or more double bonds (unsaturated) between the carbon atoms of the chain. Thus oleic acid, the commonly occurring unsaturated acid of fats, has the formula:  $\text{CH}_3 \cdot (\text{CH}_2)_7 \cdot \text{CH} = \text{CH}(\text{CH}_2)_7 \cdot \text{COOH}$ , and belongs to the series  $\text{C}_n\text{H}_{2n-1} \cdot \text{COOH}$ . Other unsaturated acids, containing two, or even more, double bonds occur in the fat of the liver, heart and kidney. The unsaturated nature of these acids is shown by their combining directly with a halogen, thus becoming saturated.

**EXPERIMENT I.** Shake up some oleic acid or its alcoholic solution with dilute bromine water. The colour of the bromine disappears. Repeat with an alcoholic solution of stearic acid, when the colour of the bromine persists.

Under suitable conditions unsaturated fatty acids and fats will also combine with iodine. The proportion of iodine with which a given mixed fat will combine therefore represents the amount of unsaturated acid present. This is called the Iodine Number of the mixed fat. (See p. 300.) Common fats are made up almost entirely of varying proportions of stearin and palmitin, which are solid at ordinary temperatures, and olein, which is liquid. The more olein a fat contains, therefore, the lower will be its melting point and the higher its iodine number.

All the fatty acids possess one property in common, viz. that they form salts. These salts are called *soaps*. By boiling neutral fat with caustic alkali, it is split up (by a process of hydrolysis) into its constituents, the glycerol being set free and the fatty acid uniting with the alkali to form a soap. This process is called *saponification*.

**EXPERIMENT II.** *Saponification of Neutral Fat.*—Place about 50 c.c. of strong caustic soda in a dish, and add about 10 grammes of fat. Heat to near the boiling-point and stir the mixture frequently. When all the fat has disappeared allow the mixture to cool. The soap forms a jelly or cake, and can be washed in cold water to remove any excess of caustic soda. A hard soap is formed if caustic soda is used; but with caustic potash a soft soap is obtained.

**EXPERIMENT III.** *Separation of Fatty Acid from Soap.*—Place about 40 c.c. of 20 per cent. sulphuric acid in a small beaker, and heat it nearly to boiling-point; drop into this pieces of the washed soap, stirring with a glass rod between each addition. The acid displaces the alkali from its combination with the fatty acid, and the latter separates out on the surface of the water as an oily layer.

**EXPERIMENT IV.** *Reactions of Fatty Acids.*—Remove some of the fatty acid with a clean glass rod, and place it on a piece of ordinary paper; a greasy stain will result.

In order to purify the fatty acid allow the contents of the beaker to cool, when the fatty acid will solidify and can be easily removed with a penknife, and transferred to distilled water in a small beaker. This removes a great part of the adherent sulphuric acid. But to free it completely it is necessary to dissolve the fatty acid in alcohol, and pour the resulting solution into excess of cold distilled water. The fatty acid which separates is filtered off and washed with distilled water. Use the purified fatty acids for the following reactions:—

A. Demonstrate that fatty acid is *acid* in reaction. For this purpose place some alcohol in a test tube, add a few drops of an alcoholic solution of phenolphthalein (an indicator which turns red with alkali, but is colourless with acids), and then a few drops of weak  $\frac{N}{10}$  caustic soda. Warm the resulting red solution on the water-bath, and drop into it small pieces of fatty acid. The red colour will disappear. Repeat the experiment with a piece of neutral fat; the result is negative.

B. Add a piece of fatty acid to some half-saturated solution of sodium carbonate, and warm; the fatty acid dissolves, carbon dioxide is liberated, and a solution of soap is obtained. Neutral fat is insoluble in cold sodium carbonate solution.

C. Press out some fatty acid between filter paper until it is dry, and apply the acrolein test as described in Experiment V. (see below). The result is negative.

D. To a solution of soap add: (a) a few drops of a solution of calcium chloride—a white precipitate of a calcium soap falls down; (b) some lead acetate solution—a white precipitate of lead soap falls down (lead plaster).

The fatty acids prepared by the above method mainly consist of a mixture of palmitic, stearic and oleic. To separate these from one another, advantage is taken of the fact that they differ in the readiness with which they form salts (soaps) with lead acetate (p. 299).

Besides these reactions of the fatty acid produced from it, neutral fat gives an important reaction, depending on the glycerol which it contains. This is called the *acrolein reaction*.

EXPERIMENT V. Place a small piece of fat in a thoroughly dried test tube, add to it three or four times its bulk of acid potassium sulphate,<sup>1</sup> and heat. A pungent vapour of acrolein<sup>2</sup> is given off, which blackens a piece of filter paper which has been dipped in ammoniacal silver nitrate solution. This reaction demonstrates that the vapour acts as a reducing agent.

*Emulsification*.—When oil is mixed with water it floats to the surface, but when a soap is present in solution in the water the oil globules remain suspended, and an emulsion results. This is more permanent if some suspending medium such as mucilage be added.

EXPERIMENT VI. In one test tube (a) place some soap solution; in another (b), some water. To each add some neutral olive oil and shake. Allow to stand, and note that a remains emulsified, b does not.

EXPERIMENT VII. Place some rancid oil (i.e. containing free fatty acid) in a test tube, add some weak caustic potash solution and shake; an emulsion forms, soap being formed by the alkali combining with the fatty acid.

EXPERIMENT VIII. Divide the emulsion produced in Experiment VII into two parts; to one of these add a little mucilage or egg-albumen and shake, and note that the emulsion persists much longer than that to which no suspending medium has been added.

### Lipoids

In addition to the neutral fats there exists in the different tissues in very varying amount a series of substances, many of them ill-defined, which possess many of the characteristics of fat and to which the name lipid has been given. These lipoids have not

<sup>1</sup> Commercial acid potassium sulphate is often impure and gives a pungent reducing vapour by itself. It is well, therefore, to make a preliminary test with the crystals alone. The impure salt can be readily purified by crystallisation.

<sup>2</sup> Acrolein is the aldehyde of allyl alcohol and has the formula  

$$\text{CH}_2 = \text{CH} - \text{CHO}.$$

all like solubilities in the various solvents used in extraction. Some are soluble in hot alcohol but not in cold, some in ether and not in acetone, and so on. These varying solubilities are used for the separation and isolation of these lipid substances.

Lipoids have been classified in various ways, and probably the simplest is

(1) *Phosphatides*. Substances which contain both phosphorus and nitrogen in varying amount. These substances are sometimes called Phospho-lipins.

(2) *Cerebrosides*. Substances which contain nitrogen but no phosphorus. These substances are sometimes called Galactosides or Galacto-lipins.

(3) A class of substances which, like neutral fat, contains neither phosphorus nor nitrogen.

Group I, the phosphatides, has been subdivided by many workers according to the N : P ratio, but recent work would show that at present only three phosphatides can be definitely identified, viz. lecithin, cephalin and sphingomyelin.

**Lecithin** is a compound of a base cholin, phosphoric acid, glycerol, and two fatty acids. It is found widely distributed in the various tissues. It is an extremely labile substance. Many functions have been ascribed to it.

**Cephalin** contains glycerol, phosphoric acid, two fatty acids and a base aminoethanol. It is mainly found in connection with nerve tissue. Both lecithin and cephalin are extracted by ether, but they are differentiated by the fact that lecithin is soluble in alcohol whereas cephalin is insoluble.

**Sphingomyelin** contains no glycerol but phosphoric acid and two fatty acids are present in addition to two bases, cholin and sphingosine. It is found in largest amount in brain tissue.

Group II, the cerebrosides or galactosides, have been much less fully studied than the phosphatides. They are the principal constituents of the substance, formerly believed to be an entity, known as protagon, a substance which can be readily extracted from brain tissue with hot alcohol. The best-known member of the group is phrenosin (sometimes called cerebrin). The cerebrosides yield on decomposition one fatty acid, the base sphingosine and a reducing sugar, the monosaccharide galactose.

**Cholesterol**.—Cholesterol is the substance selected to exemplify Group III. Although soluble in the same solvents as fats and the lecithins, cholesterol is not a fat, but an unsaturated secondary alcohol belonging to the terpene series. The terpenes are common in plants, examples of them being camphor and turpentine.

Like the lecithins it is very widely distributed in the animal body. In the free state, it is present in the envelope and stroma of the red blood corpuscles; both as an ester, and in the free state it is present in the blood. It is also present in bile, and it may separate out from this to form calculi. A variety of cholesterol,



called isocholesterol, is found in sebum and lanolin (purified wool fat), and another coprosterol is found in the fæces.

**Preparation of Cholesterol from Gall-Stones.**—The gall-stones are finely ground and boiled with 95 per cent. alcohol. The alcoholic extract is filtered hot and allowed to cool, when crystals of cholesterol separate out and can be filtered off, preferably with suction, using a perforated porcelain plate fitted in a glass funnel and covered with a disc of filter paper. The crystals are washed with a little cold alcohol, and may be re-crystallised from hot alcohol.

Cholesterol is recognised by a number of colour reactions, of which the most important are the following:—

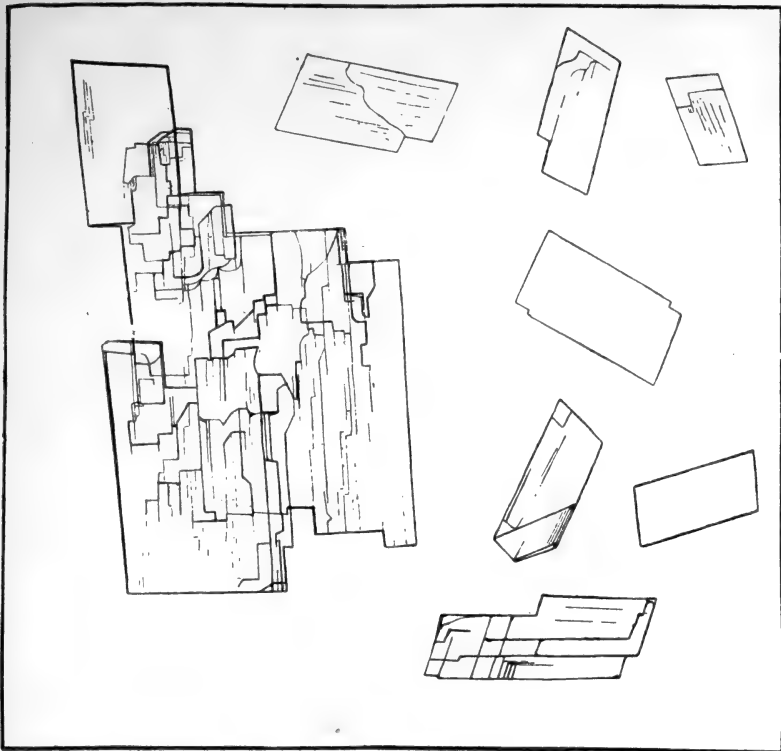


FIG. 176.—Crystals of cholesterol magnified 300 diameters.

**EXPERIMENT IX.** Place some cholesterol crystals on a microscopic slide and distribute them with a glass rod, and examine under the microscope; or better, dissolve some in absolute alcohol, place a drop of the solution on a slide, and allow it to evaporate. The crystals are colourless, glancing rhombic plates having usually a square piece removed from one corner (Fig. 176). The crystals give distinctive colour reactions.

Cover the cholesterol crystals with a cover slip and allow a

drop or so of a mixture of 5 parts sulphuric acid (conc.) and 1 part of water to run under the cover slip. Note that the edges of the crystals become red. Now run in a drop of iodine solution, when it will be noted that a play of colours results (brown, violet, blue, etc.).

Other colour reactions can be obtained with solutions of cholesterol.

**EXPERIMENT X.** Dissolve some cholesterol crystals in a few c.c. of anhydrous chloroform, and add an equal volume of sulphuric acid (conc.). Shake gently. On settling, it will be seen that the chloroformic solution becomes coloured blood red and afterwards purple, and the sulphuric acid shows a green fluorescence. If the chloroform solution be moistened with water, as by pouring it into a moistened test tube, the colour disappears. (Salkowski's reaction.)

**EXPERIMENT XI.** Dissolve some cholesterol in anhydrous acetic anhydride, and, after cooling, add some sulphuric acid (conc.). A play of colours results. (Liebermann's reaction.)

## CHAPTER VIII

### DIGESTION IN THE MOUTH AND STOMACH

#### Saliva

The salivary glands—parotid, sublingual and submaxillary—along with the numerous isolated gland acini scattered in the buccal mucosa, pour into the mouth a secretion known as saliva. The composition of this mixed saliva is as follows:—

Water . . . . .	99.42 per cent.
Organic matter . . . . .	0.36 „
Mucus and epithelial cells. Ptyalin and soluble protein.	
Potassium sulphocyanide (KCNS).	
Inorganic matter . . . . .	0.22 „
Chlorides, phosphates, and carbonates of alkalies and alkaline earths.	

It is, therefore, a very dilute secretion (specific gravity about 1,005).

The total secretion during twenty-four hours amounts to about the same as that of the urine, i.e. 1,500 c.c.

Collect some saliva in a test tube,<sup>1</sup> and perform the following reactions with it:—

#### I. To Identify the Various Constituents.

**EXPERIMENT I.** Place a drop of saliva on red litmus paper; a blue stain results. The reaction may, however, become acid where decomposition is taking place in the mouth, as is the case in decaying teeth.

**EXPERIMENT II.** Place a drop of saliva on a slide, cover and

<sup>1</sup> The secretion of saliva may be stimulated by inhaling acetic acid through the mouth, or by chewing rubber.

examine under the microscope: two kinds of cells will be seen, viz. (1) large, flat, squamous cells, which have been desquamated from the surface of the stratified epithelium of the mouth; (2) small round cells like leucocytes, which come either from the salivary glands or from the tonsils.

**EXPERIMENT III.** Place some saliva in a test tube and dilute it with an equal quantity of water; now add a few drops of 10 per cent. acetic acid, when a stringy precipitate of mucus will form. Filter off this precipitate, and note that the filtrate is watery, showing that the viscid character of saliva is due to the mucus which it maintains. To the filtrate add a few drops of Millon's reagent and boil. The result shows the presence of protein.

**EXPERIMENT IV.** Add to some saliva in a test tube a drop of a weak solution of ferric chloride (Liq. Ferri Perchlor. B.P.) and a drop of hydrochloric acid. A red colour is sometimes produced. This is due to the production of ferric sulphocyanide by interaction between the ferric chloride and a sulphocyanide which is contained in saliva. The red colour is discharged by adding a few drops of a solution of mercuric chloride (1-1,000). A more sensitive way of performing this test is to place a drop of saliva at one end of a piece of filter paper, and then to allow a drop of ferric chloride solution (acidified with HCl) to spread to the edge of the saliva drop; a deep red stain will result where the two moistened areas meet.

**EXPERIMENT V.** If some saliva be allowed to stand for an hour or so, it becomes milky or a thin surface film forms on it. This is due to the precipitation of calcium carbonate, which exists in fresh saliva in a soluble state as calcium bicarbonate. On standing exposed to the air, however, carbonic acid gas is given off, in consequence of which the bicarbonate changes into carbonate, which is insoluble. A similar precipitation of calcium carbonate, carrying with it a certain amount of calcium phosphate, sometimes occurs in the ducts of the glands and leads to the formation of calculi, or it may form on the teeth, where it leads to the formation of *tartar*.

## II. To Study the Action of the Ferment Ptyalin.

**EXPERIMENT VI.** Place a few cubic centimetres of a 0.5 per cent. solution of starch in two test tubes, *a* and *b*. To *b* add about an equal amount of saliva, and place both *a* and *b* in the water-bath at 37°-40°. Note that in a very few minutes the solution in *b* loses its opalescence and becomes clear. By means of glass rods transfer drops from each solution, about once a minute, to a white slab or dry evaporating dish, and add to each drop a little iodine solution. In the drops from the test tube *b* the blue colour becomes at first purplish and then reddish brown, and ultimately disappears. When this stage has been reached, apply Trommer's or Fehling's test to the contents of the test tube, and note that reduction occurs. In the case of *a* the blue colour persists throughout and reduction of cupric salts does not occur.

What has occurred in *b* is that the ptyalin has hydrolysed the

polysaccharide starch (blue with iodine and no reducing power), first into a simpler polysaccharide dextrin (red with iodine, no reducing power), and then into the disaccharide maltose (no colour with iodine, reduces cupric salts).

The first effect of ptyalin on starch is to convert it into so-called soluble starch (sometimes called amylo-dextrine). This gives a clear solution with water and a blue colour with iodine. Then erythro-dextrine giving a red brown colour with iodine and finally achroö-dextrine which gives no colour with iodine is formed. During each step in the breakdown a certain amount of maltose is formed. This is small in amount at first, but becomes progressively more and more with each successive dextrine formed.

**EXPERIMENT VII.** Place some 0.5 per cent. solution of starch in the mouth. After about two minutes transfer it to a test tube and test its reducing power. Repeat this experiment with a suspension of unboiled starch.

**EXPERIMENT VIII.** Repeat experiment VI (1) with saliva which has been boiled; (2) with saliva and starch kept in a test tube surrounded with ice; (3) with saliva and starch in a test tube immersed in boiling water; and (4) with saliva and starch to which a drop of mineral acid or strong caustic soda has been added. (These same experiments may be repeated with pepsin and trypsin.)

### Gastric Juice

**Acidity of Gastric Juice.**—In marked contrast to most of the other fluids of the animal body, gastric juice has a strong acid reaction towards all indicators. In disease alterations may take place in the degree and nature of the acidity of gastric juice and these variations may have a diagnostic value.

Acidity from a chemical standpoint is invariably due to the presence of excess of hydrogen ions in the solution. For the presence of these hydrogen ions, one or other of three general causes may be responsible, viz. the presence of free mineral acid, free organic acid, and acid salt. The acidity in each case is in direct proportion to the dissociation of the acid in watery solution, being greatest for mineral acid. One of the first questions, therefore, to be answered is: to which of the above causes is the presence of hydrogen ions in gastric juice due? The question is most simply answered by the use of indicators, for it has been found that the behaviour of these varies with the nature and cause of the acidity. (See also p. 338.)

Is the acidity due to a *free acid* or to an *acid salt*? Congo red is the most useful indicator for this purpose.

**EXPERIMENT I.** To a 0.2 per cent. HCl solution add a few drops of congo red solution:<sup>1</sup> the red turns to blue. Repeat with a dilute solution of acid sodium ( $\text{NaH}_2\text{PO}_4$ ) phosphate—no blue

<sup>1</sup> *Congo red solution*—dissolve 0.5 gm. of congo red in 100 c.c. of 10 per cent. alcohol.

colour develops. Show that the latter solution reacts acid towards litmus or phenolphthalein. Repeat this experiment, using, instead of a congo red solution, pieces of congo red paper prepared by dipping filter paper in a congo red solution and drying.

The result with congo red indicates that the acidity is due to free acid, and that it is almost certainly mineral acid as organic acids, except in very strong solution, do not affect congo red.

To trace further the cause of the acidity, use is made of several indicators whose behaviour towards dilute organic and combined mineral acids is quite different from that occurring in the presence of free mineral acid. The most important of these indicators are employed in the following experiments which should be performed

with 0.2 per cent. hydrochloric acid solution,  $\frac{N}{100}$  hydrochloric acid

solution (0.0365 per cent.),  $\frac{N}{10}$  lactic acid solution (0.9 per cent.) and

$\frac{N}{100}$  lactic acid solution (0.09 per cent.).

**EXPERIMENT II. Günzberg's Test.**—Place a few drops of the reagent (a solution of 2 parts phloroglucin and 1 part vanillin in 30 parts 95 per cent. alcohol) in an evaporating basin, and add a few drops of the liquid to be tested. Slowly evaporate to dryness. With dilute hydrochloric acid a red colour develops, with lactic acid no colour.

**EXPERIMENT III. Töpfer's Test.**—Add 1–2 drops of the dimethylaminoazo-benzol reagent<sup>1</sup> to some of the solution to be tested. If this contain free mineral acid a pinkish red colour develops. Organic acids, even when quite dilute, will also give a faint red colour with this reagent.

It will be found, as a result of these experiments, that the reactions obtained with the hydrochloric acid solutions resemble those of the stronger lactic acid solution, except in the case of Günzberg's reaction. This reagent gives a positive result with hydrochloric acid diluted to 1 in 10,000 parts. The Töpfer reaction with 0.2 HCl is also quite distinguishable from those given by lactic acid solutions of the above strengths, but in greater dilutions of HCl the distinction is by no means so definite.

If the contents of the stomach (removed by a stomach tube, Einhorn method or through a fistula) be tested with any of the above reagents some three hours after an ordinary meal, results like those obtained with the HCl solutions will be observed. This is taken as evidence of the presence of free hydrochloric acid.

Although in certain diseases where there is deficient secretion of hydrochloric acid, if the reaction of the stomach contents be tested, it will be found strongly acid to litmus, and if, moreover, the degree of this acidity be estimated (by the method described

<sup>1</sup> Dissolve 0.5 gram. dimethylaminoazo-benzol in 100 c.c. 95 per cent. alcohol.

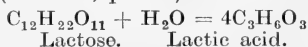
below), it may be found even higher than that of normal gastric contents. By the application of the indicator tests (especially Günzberg's), it can readily be shown that the acidity is not due to free hydrochloric acid. This leaves, as its possible causes, hydrochloric acid combined with protein, acid salts, and organic acids. Since it is known that bacteria may produce *organic acids*, especially lactic acid, this acid should be looked for.

A simple presumptive test for the presence of *lactic acid* especially in the absence of hydrochloric acid is that of Uffelmann. Add to about 5 c.c. of Uffelmann's reagent (50 c.c. of 4 per cent. carbolic acid to which a drop of liq. ferri perchloridi is added) a drop or two of the solution suspected to contain lactic acid. If it be present the violet coloured Uffelmann reagent turns canary yellow. Hydrochloric acid discharges the violet colour. If both lactic and hydrochloric acids be present, they must be separated by shaking the solution, or gastric contents (5 c.c. with 30 c.c. ether) with ether in a separating funnel. Lactic acid is soluble in ether whereas hydrochloric acid is not. If the yellow colour is still given by the ethereal solution, it may be presumed that lactic acid is present. This can be confirmed by the thiophene test.

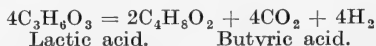
A much more sensitive and characteristic test for lactic acid is the thiophene test of Hopkins', which is applied as follows:

EXPERIMENT IV. Mix some of the water-free ethereal extract of stomach contents with 5 c.c. concentrated sulphuric acid, and transfer to a dry test tube. Add 3 drops of a saturated solution of copper sulphate, mix, heat the mixture in a boiling water bath for two minutes. Cool under the tap, and add 2 drops of a 0.2 per cent. alcoholic solution of thiophene and shake. Replace the tube in the boiling water bath. A cherry red colour will develop if lactic acid is present. Examine the solution frequently during the heating as prolonged heating causes the solution to become very dark.

The lactic acid is produced by the action of the *bacillus acidi lactici* and other organisms on sugars (see Milk, p. 328).



The fermentative process seldom stops at the production of lactic acid. Other bacteria act on the lactic acid and produce butyric acid, carbon dioxide gas, and hydrogen.



These gases accumulate in the stomach, causing flatulence. The presence of butyric acid usually reveals itself by the odour of the gastric contents. When its presence is doubtful, boil a portion of the fluid and hold a strip of blue litmus paper in the steam. If this turns red, it indicates a volatile acid (butyric or acetic). Butyric acid has a characteristic odour.

Acid phosphates ( $\text{NaH}_2\text{PO}_4$ ) when present in gastric contents are demonstrated by mixing calcium carbonate with a portion of the fluid. If an acid reaction still remains towards litmus paper, acid phosphates must be present, since the calcium carbonate will have combined with the free acids.

In the *clinical examination of the stomach contents* numerous methods have been introduced for the purpose of estimating the *total acidity*, the *total amount of hydrochloric acid*, and the *amount of free (uncombined) hydrochloric acid* contained therein. A brief outline of one of the most useful is given below.

1. **Total Acidity.**—A measured quantity (10 c.c.) of filtered gastric contents is mixed in an Erlenmeyer flask with ten times its bulk of distilled water. Two

or three drops of a solution of phenol-phthalein are added, and the solution is titrated with  $\frac{N}{10}$  caustic soda solution until a faint pink colour is just obtained. The number of c.c. of alkali required is noted.

2. **Free Hydrochloric Acid.**—Titrate another 10 c.c. sample as above using 4 drops of a 0.5 per cent. alcoholic solution of dimethylaminoazo-benzol as indicator. The end point is reached when the red colour changes to yellow.

3. **Total Hydrochloric Acid.**—Titrate still another 10 c.c. sample of the filtered gastric contents using 3 drops of a 1 per cent. aqueous solution of alizarin red as indicator. The end point is the change of the yellow colour to red violet.

Phenolphthalein titration (1) gives the total acidity of the juice.

Dimethylaminoazo-benzol titration (2) gives the free hydrochloric acid.

Alizarin red (3) reacts with all but the combined acidity.

Therefore :

1 = total acidity.

2 = free hydrochloric acid.

3 = all acidity less combined.

(1-3) = combined hydrochloric acid.

(1-2) = combined hydrochloric acid, organic acids and acid salts.

(1-2)-(1-3) = organic acids and acid salts (probably chiefly acid phosphates).

The number of c.c.  $\frac{N}{10}$  NaOH required for each 10 c.c. of gastric juice for neutralisation multiplied by 0.0365 (as 1 c.c.  $\frac{N}{10}$  alkali equals 0.00365 gm. HCl), gives the acidity for 100 c.c. gastric juice in grms. HCl.

Total hydrochloric acid may also be determined as follows :—10 c.c. of filtered gastric contents are placed in a platinum dish and evaporated to dryness on the water bath. The dish is then heated to a low red heat, so that charring is complete, but the resulting carbonaceous material is not burnt up. The mineral chlorides alone now remain in the dish. The contents of the dish are rinsed with hot distilled water through a funnel into a 100 c.c. measuring flask. The flask is cooled, 5 c.c. nitric acid and 20 c.c.  $\frac{N}{10}$  silver nitrate solution are added, and the contents made up to 100 c.c. The amount of silver nitrate used in precipitating the chloride present is then determined by Volhard's method (see p. 288). A similar experiment is performed with the same volume of the gastric contents, to which slight excess of sodium carbonate solution is added before evaporation, and again the amount of silver nitrate used in precipitating the chloride determined. The first experiment gives the mineral chloride present, equivalent, say, to 5 c.c.  $\frac{N}{10}$  silver nitrate. The second experiment gives the total chloride, equivalent, say, to 10 c.c.  $\frac{N}{10}$  silver nitrate. The difference gives the [volatile chloride, that is, the hydrochloric acid free and combined with protein. In the hypothetical case this is  $10-5 = 5$  c.c.  $\frac{N}{10}$  silver nitrate. The gastric contents, therefore, contain  $\frac{0.365 \times 5}{10}$  per cent. total hydrochloric acid.

Normal human gastric contents obtained after a meal containing very little protein usually contain about 0.2 per cent. total hydrochloric acid. This hydrochloric acid determination is of value, as it is the best measure of the secretory activity of the gastric mucous membrane in pathological conditions.

**The Organic Matter.**—If pure gastric juice be cooled to 0° C., a precipitate falls down. On analysis, this precipitate is found to have nearly the same percentage composition as protein; and on testing its action on a solution of protein, it is found to contain pepsin. Pepsin of similar composition can also

be prepared by saturating gastric juice with ammonium sulphate, which precipitates it. The methods employed for obtaining ferment from the gastric mucosa after death yield a still more impure product, on account of the ferment adhering to the proteoses, etc., which are always present in the final precipitate.

Prior to its secretion, pepsin exists in an inactive form as granules in the gland cells of the stomach mucosa. This precursor or zymogen is called pepsinogen. It differs from pepsin in that alkali does not destroy it, whereas alkali destroys pepsin.

The most favourable conditions for the action of pepsin may be studied in the test tube as described in the following experiments:—

**The Action of the Gastric Juice.**—The most convenient protein for studying the action of pepsin is blood fibrin which has been very thoroughly washed with boiling acidulated water so as to remove all impurities. Cubes of coagulated egg white may also be employed, but they digest more slowly than fibrin.

EXPERIMENT V. Label six test tubes *A, B, C, D, E, F*, and place a small piece of fibrin in each. Half fill *A* with water, *B* with 0.2 per cent. HCl, *C* with water and a few drops of peptic extract, *D* with 0.2 per cent. HCl and a few drops of peptic extract,<sup>1</sup> *E* same as *D*, but boil the mixture, and *F* with 1 per cent. sodium carbonate solution and a few drops of the peptic extract.

Place all these in a water bath kept at body temperature (37–38°). Observe that in *A* and *C* the piece of fibrin remains unchanged, whereas in *B, D*, and *E*, which all contain 0.2 per cent. HCl, it becomes swollen and transparent. In *F*, which contains alkali, it does not swell.

EXPERIMENT VI. After about half an hour, remove a sample of the contents of any of the tubes containing acid, colour it faintly with a drop or two of litmus solution, and then carefully neutralise with weak sodium carbonate solution (1 part 1 per cent. sodium carbonate + 2 parts of water). A precipitate of acid meta-protein is usually produced (for reactions, see proteins, p. 202).

The first stage in gastric digestion of proteins consists, therefore, in the production of acid meta-protein by the weak HCl.

EXPERIMENT VII. Remove a sample of the contents of *D* and apply the following tests: (a) The Biuret reaction—rose-pink colour; (b) Add nitric acid (conc.)—white precipitate, which clears up on heating and returns on cooling; (c) Add a few drops of a saturated solution of salicyl-sulphonic acid. A white precipitate results which disappears on heating and returns on cooling. These results show us that proteoses have been produced (see p. 203).

Gastric juice can also curdle milk. This action is usually attributed to the ferment rennin, but it is probable that rennin and pepsin are identical, as proteolytic ferments always have a rennin action, and the proteolytic activity of a given ferment is proportional to its rennin activity.

EXPERIMENT VIII. Prepare five test tubes, *a, b, c, d, e*. Into

<sup>1</sup> Use larger quantities of fibrin and fluid in this test tube, because the products of digestion will be required for succeeding experiments.



all put about 5 c.c. milk, then to (a) add 5–10 drops of a solution of rennin, to (b) 5–10 drops rennin which has previously been boiled, to (c) 5–10 drops rennin + 5 drops 0.2 per cent. potassium oxalate solution, to (d) 5–10 drops rennin + 5 drops 0.2 per cent. potassium oxalate solution + 3–4 drops 5 per cent. calcium chloride solution, and to (e) 5–10 drops rennin + 5 drops 0.2 per cent. potassium oxalate solution, after standing ten minutes heat to boiling, cool and add calcium chloride solution. Shake test tubes a, b, c, d well and place in a water bath at 40° C. for five to ten minutes. Tube e treat as directed.

Tube (a) clots, (b) no clotting, (c) no clotting, (d) clots, (e) clots. Rennin and calcium salts are therefore necessary for the curdling of milk. Further, although rennin alone cannot clot milk, yet as test tube e shows it produces some change in the caseinogen which is completed by the addition of a calcium salt (see Milk, p. 328).

## CHAPTER IX

### DIGESTION IN THE INTESTINE

**Pancreatic Digestion.**—In studying the digestive action of this juice we may employ, as in the case of gastric digestion, an extract of the gland. This extract may be made with glycerine, or preferably water, after activation of the zymogens present in the minced tissue by means of the addition of a small amount of intestinal mucosa scrapings.

There are three active ferments in pancreatic juice, one proteolytic—trypsin; one amylolytic—amyllopsin or diastase; one lipolytic—steapsin or lipase.

**Trypsin.**—Like pepsin, this ferment hydrolyses protein, and leads to the production of proteoses and peptones. In this case, however, digestion is more complete. Under suitable conditions the proteoses and peptones can disappear entirely, polypeptides and amino acids resulting; the ultimate decomposition products are, in fact, almost the same as when a strong acid is used as the hydrolysing agent (see Proteins, p. 193).

**EXPERIMENT I.** A solution of pancreatic extract in 1 per cent. sodium carbonate is prepared (Liq. Pancreaticus (Benger), diluted thirty times with 1 per cent. sodium carbonate solution). In order to study the action of this on proteins, add to it a piece of fibrin which has been soaked over night in 1 per cent. sodium carbonate solution, and place in a water-bath at body temperature.

The following points of difference may be noted between this and the peptic digestion of fibrin: (1) The reaction is alkaline; (2) there is no preliminary swelling of the fibrin; it is gradually eaten away (erosion); (3) when the piece of fibrin has nearly disappeared remove a sample of the digest, and neutralise with weak

acetic acid. A precipitate of alkali meta-protein results (for Reactions, see p. 202).

Apply to another sample the tests for proteoses and peptones, and note that they are positive.<sup>1</sup>

**EXPERIMENT II.** If the pancreatic extract in Experiment I be boiled before the fibrin is added, no digestion will result. The digestive agent is, therefore, a ferment which is destroyed by heat.

**EXPERIMENT III.** Repeat Experiment I, making the reaction acid by means of hydrochloric acid. Note that, although the fibrin becomes swollen up—as this depends on the acid, not on the ferment—no formation of proteoses or peptone occurs. The trypsin cannot act in acid medium, being destroyed in this reaction.

Trypsin can carry digestion further than pepsin. Allow a digest to go on for twenty-four hours or longer. Filter and evaporate to small bulk some of the filtrate. Take a drop of this concentrated filtrate and allow it to dry on a microscopic slide; examine the crystals of leucine and tyrosine present, noting that the former consist of round balls frequently with concentric markings and yellowish in colour, whereas the latter are usually grouped into sheaves of needles (see Fig. 177). Test the filtrate for protein by the biuret test. Also test it by adding some drops of chlorine or bromine water. Note the red colour which results due to the presence of tryptophan.

**Diastase.** The old name for pancreatic diastase is *Amylopsin*. This ferment acts on starch in a way similar to ptyalin, i.e. it converts starch into dextrines and maltose. It can act on unboiled starch.

**EXPERIMENT IV.** Take four test tubes *a, b, c* and *d*. Into *a* place 5 c.c. of a suspension of starch; into the other three tubes put 5 c.c. of boiled starch solution. Add to *a, b* and *c* a few drops of pancreatic extract (glycerine extract is good) and to *d* a similar amount of boiled pancreatic extract. Add to tube *c* in addition a trace of sodium chloride. Place all four tubes in a water-bath at 40° C. Remove drops from time to time and test on a porcelain slab with iodine. When all colour disappears, test contents of the tubes by Fehling's test.

**Lipase.**—The old name for pancreatic lipase is *Steapsin*. This ferment breaks down neutral fat into fatty acids and glycerol (see p. 222).

**EXPERIMENT V.** Take two large test tubes and into each place about 10 c.c. of neutral cream (i.e. cream to which a few drops of an alcoholic solution of phenolphthalein has been added, and then  $\frac{N}{10}$  NaOH until a definite red colour appeared). Add to one test tube a few drops of fresh watery pancreatic extract and to the other a few drops of boiled extract; then make the contents definitely

<sup>1</sup> No primary proteose is formed by tryptic digestion; there is, however, a considerable amount of secondary proteose (see p. 203).

alkaline, if necessary, by a further addition of  $\frac{N}{10}$  NaOH. Place both tubes in a water-bath at  $40^{\circ}$  C. and note rate at which the red colour disappears due to the liberation of fatty acid. This method may be made roughly quantitative by noting the amount of alkali required to neutralise the fatty acid formed.

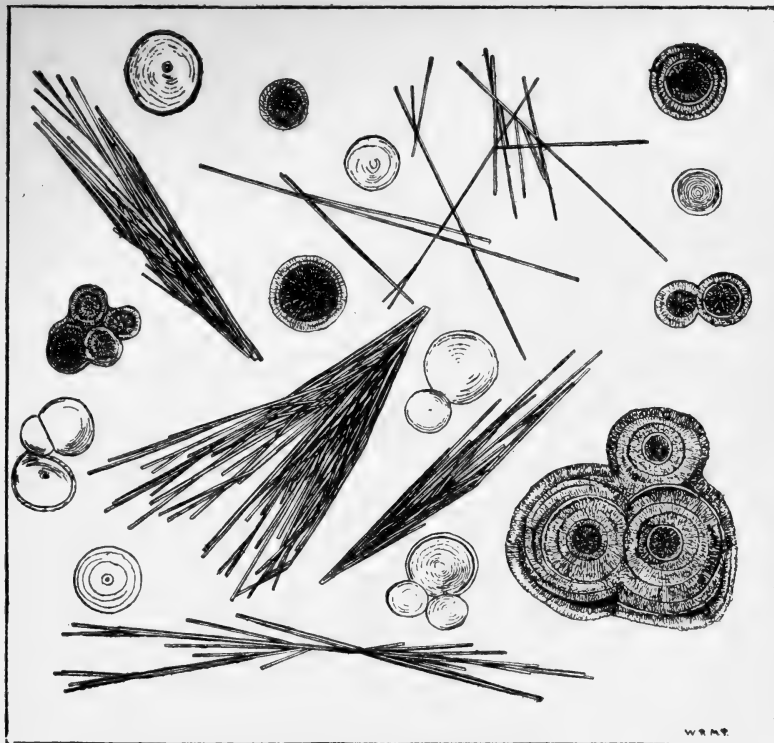


FIG. 177.—Crystals of leucine and tyrosine.

## CHAPTER X

### THE BILE. BACTERIAL DIGESTION

**Composition of Human Bile.**—In I the bile was obtained from the gall bladder: in II the bile was obtained from a fistula.

	100 parts contain—	I.	II.
Water . . . . .		83	97
Solids . . . . .		17	3
Bile salts . . . . .		9	1.0
Mucin and bile pigment. . . . .		3	0.5
Cholesterol . . . . .		0.9	0.26
Lecithin and fat . . . . .		0.5	1.2
Inorganic salts . . . . .		0.8	0.7-0.8

Besides these, bile also contains traces of soaps, fats and urea. Compounds of glycuronic acid have also been found in bile.

**EXPERIMENT I.** Examine some ox bile. Note that it has a greenish colour, a peculiar musk-like odour, a bitter-sweet taste, a faint alkaline reaction to litmus paper, and that it is of a slimy consistency.

**EXPERIMENT II.** If a few drops of weak acetic acid be added to a few cubic centimetres of bile, a stringy precipitate is produced. This consists in certain animals (ox) of nucleo-protein, in others (man) of mucin. Filter off this precipitate, and note that the filtrate has lost its slimy character. Boil the filtrate; no coagulum is produced, therefore bile contains no native protein.

So far as can at present be ascertained, the amounts of pigment and of bile salts do not bear a quantitative relationship to one another, so that it is improbable that they are both derived from the same source.

**The Chief Bile Salts** are two in number, glycocholate and taurocholate of sodium. The two acids are closely related as on hydrolysis both yield cholalic acid. In the first instance it is combined with aminoacetic acid—glycine, in the other with aminoethyl-sulphonic acid—taurine.

**EXPERIMENT III.** Test another portion of the bile for bile salts by *Pettenkofer's reaction*. To do this place a drop of bile in a small evaporating dish, and move this about so that a thin film of the bile is produced. Now add to the film a very small drop of a concentrated watery solution of cane sugar, and then a few drops of concentrated sulphuric acid. A purple colour is produced, which can be intensified by warming. Or it may be more simply carried out by shaking a dilute bile solution to which a small quantity of cane sugar solution is added until the test tube is half full of froth. A few drops of concentrated  $H_2SO_4$  are carefully run down one side of the test tube: froth at place of contact becomes purple.

This pigment shows absorption bands in the spectrum. The chemistry of this reaction is that the sulphuric acid acts on the cane sugar to produce a body called furfuraldehyde, which then reacts with the cholalic acid of the bile salts to produce the pigment. Where only traces of bile salts are present, the test may be made more delicate by using a solution of furfuraldehyde (1 in 1,000) instead of cane-sugar.

**EXPERIMENT IV.** *Hay's Sulphur Test*.—If a small pinch of powdered sulphur be sprinkled on the surface of bile, or of a solution containing bile salts, it will sink to the bottom of the vessel; whereas with most other fluids it remains floating on the surface. This reaction depends on the fact that bile salts lower the surface tension of fluids in which they are dissolved. For comparison repeat this test with water.

**The Bile Pigments** are bilirubin and biliverdin. The former is most abundant in bile of carnivorous, and the latter in that of herbivorous animals. They are detected by *Gmelin's test*, the play

of colour following the gradual oxidation of the pigments by means of nitrous acid.<sup>1</sup>

**EXPERIMENT V.** Dilute some ox bile with an equal volume of water. Hold the test tube as nearly horizontal as possible, and allow some fuming nitric acid to run down it, so that this forms a layer under the bile. Where the two fluids are in contact, a play of colours is produced. This test can be rendered still more delicate by filtering a little diluted bile through white filter paper, then removing and opening out the filter paper and placing a drop of fuming nitric acid on it.

Bilirubin is the least oxidised bile-pigment, and its empirical formula is  $C_{32}H_{36}N_4O_6$ . If we compare this with the formula of hæmatin— $C_{32}H_{32}N_4O_4Fe$ —we see that it must be from this body that it is derived, the change being the abstraction of iron and the addition of two molecules of water. This is also the formula of iron-free hæmatin or hæmatoporphyrin, and of hæmatoidin, a pigment which crystallises out in old blood clots in the tissues. Although the same empirically, these bodies vary somewhat in their physical behaviour and neither of them gives Gmelin's test, so that we may assume that they have different constitutional formulæ.

**EXPERIMENT VI.** Bilirubin can be extracted from pigmented gall-stones. The gall-stones are ground to a rough powder and extracted by heating with 95 per cent. alcohol, to which a few drops of strong hydrochloric acid have been added. (The acid is necessary to decompose the compound of bile pigment with calcium present in the stones.) The coloured extract is then cooled. The crystals of cholesterol, which separate, are filtered off, washed with alcohol and examined (see p. 225). The filtered extract is placed in a dish, and pure nitric acid run in, drop by drop, when a brilliant Gmelin's test is obtained.

**EXPERIMENT VII.** Place some bile in a test tube, and add one or two crystals of cholesterol to it and gently warm. The cholesterol dissolves. Before doing this show that the crystals will not dissolve in water.

**Inorganic Salts.**—These are chiefly sodium carbonate and disodium hydrogen phosphate.

**The Uses of the Bile in Intestinal Digestion.**—(1) It is an alkaline fluid, containing a viscid substance (mucin, etc.); consequently, it assists in the emulsification of fats.

**EXPERIMENT VIII.** Shake up some rancid oil with bile in a test tube. Notice that a very stable emulsion is formed (see Fats, p. 223).

(2) It causes a precipitate when added to an artificial peptic digest.

**EXPERIMENT IX.** Add some bile to a sample of a twenty-four hours' peptic digestion of egg-white. A precipitate of proteins is produced.

<sup>1</sup> This test depends on the various colours of the oxidation products of bilirubin. The first oxidation product is biliverdin (green); the next biliverdin (blue); the next bilipurpurin (purple); and, finally, choletelin which is yellow.

**Bacterial Action.**—The conditions necessary for bacterial growth are very favourable in the intestine. As a result of their growth, bacteria decompose the food-stuffs and lead to the production of products in many cases the same as those of the digestive juices, in other cases of a different nature. In the small intestine the bacteria which are most active are those acting on carbohydrates, whereas in the large intestine these are largely replaced by bacteria acting on protein.

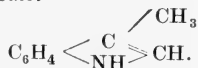
Their action on *proteins* leads to the production of proteoses, peptones, and amino acids, etc. So far their action corresponds to that of trypsin, but they digest farther and produce a multitude of simple degradation products, such as ammonia, fatty acids, carbonic acid, etc., as well as a group of substances belonging to the aromatic series.

The aromatic bodies are arranged in two groups. The one contains *phenol*  $C_6H_5OH$  and its methyl derivative *cresol*  $C_6H_4 \begin{matrix} \text{CH}_3 \\ \text{OH} \end{matrix}$ . These are produced from tyrosine, which has the formula



When this changes into cresol and phenol, the amino-propionic acid side-chain loses, first its amino group as ammonia, and then its carboxyl and methyl group are oxidised and given off as carbonic acid and water.

The other group is more complex, and contains *indol*  $C_6H_4 \begin{matrix} \text{CH} \\ \text{NH} \end{matrix} \text{CH}$  and its methyl derivative *scatol*



These are derived from tryptophan (p. 310).

Anaërobic bacteria first of all act on the tyrosine and tryptophan, and split off from them the amino ( $NH_2$ ) groups as  $NH_3$ . After this has been accomplished, aërobic organisms act on the remaining side chains yielding carbon dioxide and water.

Certain of these aromatic bodies—especially scatol—have a strong faecal odour which they impart to the faeces. Considerable proportions of them are, however, absorbed into the blood and reappear in the urine as indoxyl and scatoxyl in combination with sulphuric acid and alkalis as aromatic sulphates (see p. 264).

**EXPERIMENT X.** Preparation and reactions of Indol, Scatol and Phenol. Prepare an artificial digestion mixture with pancreatic extract, or minced pancreas, and allow it to incubate without the addition of an antiseptic, until it has an intense and disagreeable odour. The digest is then acidified with acetic acid and placed in a large flask connected with a Liebig condenser. Distillation is continued as long as the distillate has a marked odour. (Indol distils over much more quickly than scatol.) The following tests are then applied to portions of the distillate:—

**Indol.**—1. *Legal's Test*—To a few c.c. of the solution in a test tube add a few drops of sodium nitro-prusside solution and then ammonia till alkaline. A deep reddish violet colour results, which changes to blue on acidifying with acetic acid. 2. Add to a few c.c. of the liquid about 2 c.c. of each of the following solutions: (i) Para-dimethyl-amino-benzaldehyde 4 parts, 95 per cent. alcohol 380 parts, hydrochloric acid (conc.) 80 parts. (ii) Potassium persulphate 2 grams in 100 c.c. water. A reddish pink colour results.

**Scatol.**—Warm some of the solution with an equal volume of strong sulphuric acid. A red colour results.

**Phenol.**—Boil some of the solution with Millon's reagent. A red colour, but no precipitate is formed (see Estimation of Phenol in Urine, p. 291).

## CHAPTER XI

## BLOOD

To the unaided eye ordinary blood appears to be a homogeneous red fluid, but examination with the microscope shows that the red colour is confined to certain formed elements, the red corpuscles, suspended in a faintly yellow fluid, the plasma.

**EXPERIMENT.** (a) Note with the aid of a microscope the disposition of the colouring matter, the hæmoglobin, in the red blood corpuscles or erythrocytes. (b) Undiluted blood appears to be an opaque fluid. Take two test tubes, place 5 c.c. water in one and an equal volume of 0.9 per cent. sodium chloride solution in the other and then add to each a drop of whipped blood. The pigment dissolves out of the corpuscles in the first, the mixture becoming transparent (*laked*) but not in the second. Confirm by the microscope. (c) Test the Specific Gravity. Take mixtures of chloroform and benzene ranging from a specific gravity of 1,040 to 1,070, or a series of solutions of sodium sulphate of similar range of specific gravities. Introduce the blood to be tested (a drop of blood from the finger) below the surface of the fluid by means of a capillary tube. Note whether the drop floats, sinks, or remains suspended. Note the specific gravity of the solution in which the drop remains suspended. (d) The reaction of the blood may be determined by placing a freshly-drawn drop of blood on red *glazed* litmus paper, allow it to remain for a few seconds, then wash it off under the tap.

**The Clotting of Blood.**—When blood is shed it sets at first to a red jelly, after a time this jelly contracts and gradually squeezes out a pale yellow fluid, the serum.

**EXPERIMENT I.** Carefully sterilise a needle, prick the finger, and draw some blood into a fine capillary tube. Place aside and examine under the microscope at the end of the lesson.

In order to study the nature of the processes involved in the coagulation of blood, it is essential to stop clotting from taking place. This can be done in several ways, such as receiving blood into certain neutral salts (*e.g.* quarter volume of magnesium sulphate, equal volume of sodium sulphate), or on to a soluble citrate, oxalate or fluoride. Upon standing, the corpuscles will gradually sink, and the supernatant plasma can be pipetted off, or, preferably, the mixture can be centrifugalised. This plasma is called "salted" or "oxalated," or "fluoride" plasma and so on.

**EXPERIMENT II.** Take about 5 c.c. of salted plasma in each of three large test tubes, *a*, *b* and *c*, and then dilute each by the addition of 5 volumes of water. Leave *a* untouched; to *b* add a few drops of normal serum; to *c* a few drops of serum previously heated to 60° C. Place all three tubes in water-bath at 37° to 40° C. *a* and *c* clot about the same time, *b* clots more quickly, *i.e.* addition of normal serum hastens clotting.

**EXPERIMENT III.** In each of four tubes, *a*, *b*, *c* and *d*, place 5 c.c. oxalated plasma. Leave *a* untouched; to *b* add a few drops of serum; to *c* a few drops 1 per cent. calcium chloride solution; and to *d* an equal volume of saturated sodium chloride solution. Filter off the flocculent precipitate and keep for the next experiment, which should be performed as soon as possible. To the filtrate add some calcium chloride solution. Place all in a water-bath as above. It will be found that *a* has not clotted, that *b* may have clotted, *c* has clotted, and that the filtrate *d* has not clotted.

From these experiments, we gather (1) that blood will not clot when the calcium salts have been removed by an oxalate; (2) that serum can clot oxalated blood (that is, blood without the presence of calcium salts), because it contains the necessary enzyme already formed in it; (3) that oxalated blood will clot when calcium is added to it, because with free calcium available the coagulating enzyme can act; (4) that the body coagulated is a protein thrown out of solution by half saturation with sodium chloride solution. This body is known as *fibrinogen*. It is insoluble in distilled water and easily thrown out of solution by saturation with salts, and, therefore, belongs to the globulin class of proteins.

**EXPERIMENT IV.** Quickly redissolve in water the precipitate obtained in Experiment III, *d*. The salt adhering to the precipitate forms a dilute saline solution, in which the precipitate dissolves. Test the solution obtained for protein by the colour tests.

**EXPERIMENT V.** Into the bent capillary tubes provided collect some of your own blood, first introducing a small quantity of anti-coagulant fluid, preferably 10 per cent. sodium citrate, since sedimentation is most rapid with this solution; 1 per cent. potassium oxalate or 3 per cent. sodium fluoride may also be used. Having sealed off the ends, under the demonstrator's supervision, hang it upon the centrifuge by the bent end. With the plasma so obtained, perform experiments such as those given in Experiment III. Sodium citrate, it will be found, acts like oxalate. This is not because it precipitates the calcium, but because it combines with it to form a soluble citrate, a salt which does not ionise (dissociate) in solution, and therefore leaves no calcium free to aid in the formation of thrombin. If fluoride has been used, it will be found that the addition of calcium salts to the plasma does *not* cause a clot to form, showing that the fluoride in some way prevents the formation of the enzyme from the pro-enzyme.

**Conditions which retard Clotting.**—(1) *Cold*—receive the blood into a vessel placed in ice (i.e. keep it at a temperature a little above freezing point). The enzyme action is inhibited by cold. The blood clots on warming.

(2) *Contact with blood-vessel wall.*—“The living test tube.” This is made by ligaturing in two places a vein of a large animal, such as the jugular vein of the horse. In the tube thus formed the blood does not clot, and if it be hung up the corpuscles gradually sink to the bottom, leaving the unclotted plasma above.

(3) Addition of *certain neutral salts.*—“Salted plasma” (cf. Experiment II).

(4) Addition of *a soluble oxalate.*—“Oxalate plasma” (cf. Experiment III).

(5) Addition of *a soluble citrate.*—“Citrate plasma” (see Experiment V).

(6) Addition of *a soluble fluoride.*—“Fluoride plasma.” This plasma will not clot upon the addition of calcium (see Experiment V).

(7) Addition of *leech extract* (Hirudin).—This is a secretion produced by the salivary glands of the leech, and which can be obtained by extracting the heads with water. It acts because it contains an antithrombin.



(8) *Contact with oil*.—Receive the blood into a smooth vessel smeared with oil.

(9) *Intra-vitam methods*.—These consist in injecting certain substances into the blood-vessels of the animal before bleeding it. These substances are:—

(a) Commercial peptone, which consists mainly of proteoses.

(β) Soap solution.

(γ) A weak alkaline solution of nucleo-protein injected *slowly*—the so-called “*negative phase*” of nucleo-protein injection.

Peptone probably acts by causing the liver to form a large amount of *anti-thrombin*, which normally keeps blood from clotting inside the vessels. The exact action of nucleo-protein is not well understood.

**Conditions which hasten Clotting.**—(1) *Body temperature*.

(2) *The addition of some clotted blood* (clot or serum).

(3) *Agitation*, e.g. whipping the blood with a bunch of twigs. This is a very general method of keeping blood fluid when it is not desired to study the phenomena of clotting.<sup>1</sup>

(4) *Contact with a rough surface* (cf. effect of receiving into oil).

(5) *Addition of calcium salts*.

(6) *Intra-vitam methods* causing blood to clot within the vessels:—

(a) Injury or death of blood-vessel wall. When an artery is crushed, as in a contused or lacerated wound, a clot forms, which acts as a natural plug to prevent hæmorrhage. When the arterial wall undergoes degeneration a clot or *thrombus*, as it is termed, may form. Similarly, when a blood-vessel is ligatured the inner coat is injured, and a clot forms for a short distance from the ligature. This clotting is due to the liberation of thrombokinase from the injured tissues, causing the formation of some thrombin. That the clot does not extend indefinitely in the blood is due to two causes: (a) thrombin is adsorbed into the fibrin it precipitates; and (b) the formation of anti-thrombin.

(b) *Rapid injection* into a vein of a strong alkaline solution of nucleo-protein; the so-called “*positive phase*” of nucleo-protein injection.

**Preparation of Fibrin Ferment.**—Blood serum, or some defibrinated blood, is mixed with twenty times its bulk of alcohol. A copious white precipitate is obtained. Allow this to stand under the alcohol for two months. By this time all the other proteins present will be coagulated, except fibrin ferment. The fluid is pipetted off, the sediment carefully collected on a filter, and after the alcohol has drained off ground up in a mortar with water. This extracts the fibrin ferment. Filter, and keep filtrate.

### Blood Serum

**Proteins.**—EXPERIMENT VI. Divide into three portions—*a, b, c*.

(a) Allow *a* to drop gradually into a beaker filled with distilled water; a cloud forms round each drop as it mixes with the water. This is due to the precipitation of the globulin present, as there is now too little saline present to keep it in solution.

(b) Saturate *b* with crystals of magnesium sulphate; a precipitate of globulin occurs. Filter. Show that the filtrate contains albumin (i) by faintly acidifying with acetic acid and heating in a water-bath—note the temperature at which the albumin coagulates (77°–79° C.); (ii) fully saturating the solution with ammonium sulphate.

Redissolve the precipitate of globulin in water; faintly acidify and note the temperature of heat coagulation (75° C.).

(c) To *c* add an equal amount of fully saturated  $(\text{NH}_4)_2\text{SO}_4$  (*half*

<sup>1</sup> It is important to remember that this is no longer normal blood, but defibrinated blood.

saturation). The globulin is precipitated. Filter and *fully* saturate (add solid crystals) with  $(\text{NH}_4)_2\text{SO}_4$ ; a precipitate of albumin results.

**Salts.**—EXPERIMENT VII. Faintly acidify the serum and boil to coagulate the proteins. Filter. Test the filtrate for:—

(a) chlorides by silver nitrate—white precipitate insoluble in nitric acid;

(b) phosphates—white precipitate on addition of ammoniacal magnesium citrate solution. Filter off this precipitate. Dissolve in nitric acid, and heat with nitro-molybdic acid—yellow precipitate;

(c) sulphates—white precipitate with barium chloride, insoluble in hydrochloric acid.

In all three tests phosphates are precipitated, but in (a) they are soluble in nitric acid, in (c) they are dissolved by hydrochloric acid (cf. salts of urine).

The amount of sulphate present is usually very small. This filtrate may also be tested for sugar by Fehling's test.

**Blood Plasma.**—All the above bodies are present in plasma, which contains one substance in addition, namely, Fibrinogen. This has already been shown (Expt. III, *d*). Plasma, however, does not contain thrombin; serum does.

When the function of the blood is remembered it is obvious that there are many bodies other than the above present in both plasma and serum in small quantities. Thus the blood carries the food materials to the tissues, and the products of metabolism away from them. There is, therefore, in addition to ammonia, small quantities of nitrogenous extractives:—urea, uric acid, creatinine, xanthine, hypoxanthine, etc.; of non-nitrogenous extractives, fats, cholesterol, lactic and other organic acids.

### The Leucocytes or White Blood Corpuscles

These are morphologically the same as other cells, and they contain the same chemical substances. The *protoplasm* consists mainly of water. The solids consist of various proteins, which chiefly belong to the group of compound proteins (gluco-proteins and nucleo-proteins), and there is also a small amount of albumin and globulin. The protoplasm may also contain such substances as glycogen, fat, mucin, etc., which have either been produced by the activity of the protoplasm, or which are simply deposited in the cell for storage purposes.

The *nucleus* seems to consist mainly of nucleo-proteins, nuclein and nucleic acid. The nucleo-protein of the nucleus is said to contain a higher percentage of phosphorus than does that of the protoplasm.

### The Erythrocytes or Red Blood Corpuscles

Structurally these are said to consist of a **stroma** containing in its meshes a chromo-protein called **Hæmoglobin**. It is, however, impossible to demonstrate this stroma histologically, and some authori-

ties believe that the hæmoglobin is merely contained in a colloidal state in a protein envelope.

Chemically they contain about 60 per cent. of water and nearly 33 per cent. of hæmoglobin, and varying amounts of lecithin, cholesterol, nucleo-protein and salts.

**Hæmoglobin.**—This is a compound protein containing 0.4 per cent. of iron. When decomposed by acids or alkalis it splits up into a protein of the nature of a histone (see p. 198) called **globin** and into a pigment called **hæmatin**, which contains all the iron. A pure solution of hæmoglobin can be obtained by centrifugalising blood,<sup>1</sup> removing the serum with a pipette, shaking up the corpuscles with a 0.85 per cent. sodium chloride solution<sup>2</sup> (which is nearly *isotonic* for the blood of the ox, horse, or man), and again centrifugalising.

By this means the corpuscles are thoroughly washed free of serum, etc. They are then collected and treated with two or three times their bulk of distilled water, in this the hæmoglobin dissolves, a deep red solution resulting. The corpuscles have become "laked."

**EXPERIMENT VIII.** Heat carefully some hæmoglobin solution. It decomposes at about 60° C., and the protein coagulates on further heating. Also test the solution for protein; it gives several of the ordinary protein reactions, but in each case a splitting into protein and hæmatin simultaneously ensues.

Besides being dissolved out by distilled water the hæmoglobin may be set free from the red corpuscles by (i) warming to 50° C.; (ii) the addition of a little ether, or of dilute ammonia solution; (iii) the addition of bile, saponin, or the serum of another species of animal.

That hæmoglobin contains iron can be shown by the following experiment:—

**EXPERIMENT IX.** Dissolve some dried blood by heating with strong nitric acid. Evaporate nearly to dryness in a dish. Dissolve in water and add potassium sulphocyanide solution. A blood-red colour indicates the presence of iron.

**Crystals of hæmoglobin.**—These are most easily obtained from such animals as the rat or guinea-pig; with more difficulty from man and most other mammals.

**EXPERIMENT X.** Mix a drop of rat's blood with a drop of water upon a slide. After several minutes examine under the microscope for hæmoglobin crystals.

Hæmoglobin, as we have seen, is a compound protein consisting of two parts, the iron containing portion "*hæmatin*" and the protein portion "*globin*." Hæmatin has the formula  $C_{33}H_{33}N_4O_5Fe$ . It itself does not crystallise, but a compound of hæmatin with

<sup>1</sup> Horses' blood should be used for this purpose as the corpuscles sink more quickly than do the corpuscles of any other blood.

<sup>2</sup> A salt solution of this strength has the same osmotic pressure as the contents of the red blood corpuscle, and consequently no swelling or crenation of the corpuscle is produced.

hydrochloric acid and some other body (acetic acid, or an alcohol according to the method of preparation) called hæmin can be obtained from hæmoglobin, which crystallises in chocolate-brown rhombic plates. This forms one of the chemical tests for blood.

EXPERIMENT XI. *Preparation of Hæmin Crystals.*—Place a drop of blood upon a glass slide and warm until dry. Scrape loose the brown residue, add a little glacial acetic acid, cover with a cover



FIG. 178.—Hæmin.  $\times 1,500$ .

glass and warm very gently until bubbles form. Remove from flame. If necessary add a little more acid, and warm again till bubbles form. Repeat the operation two or three times. When cold examine with microscope for the dark-brown hæmin crystals (Fig. 178).

There is sufficient chloride in blood to give the test without the addition of any sodium chloride. If, however, an old blood stain be used, it is necessary to add a small crystal of sodium chloride in case the chloride of the blood has been washed out. Bromide

or iodide may be used instead of chloride, yielding a hæmin with a corresponding change in composition.

Another chemical test for blood depends upon the fact that the iron containing portion of the hæmoglobin will, in the presence of such oxidising agents as hydrogen peroxide, or old "ozonised" turpentine, convert a coloured body like tincture of guaiac (brown) to another coloured derivative (blue).

**EXPERIMENT XII.** Boil some diluted blood. Add 2 drops of tincture of guaiac (or of an alcoholic solution of guaiconic acid), then sufficient alcohol to dissolve the precipitate, and *lastly* a little ozonic ether, ozonic alcohol, or old oil of turpentine. A blue colour is formed in the presence of blood. Ascertain in what dilution blood gives this test. Ozonic ether and ozonic alcohol contain hydrogen peroxide.

The solution is first boiled to destroy any oxidising enzymes present. These bodies can effect the same change, as also can many salts of metals, such as copper, iron, gold, cobalt, strong sodium chloride solution, and various other fluids such as milk, saliva, mucus, sweat, and juices of vegetable origin (extract of pea flour, fruit juices, etc.). If, however, the solution be first boiled, in the absence of metallic salts, the reaction is to be regarded as a reliable one for blood. In any case, if the test be *negative*, most investigators regard it as certain that *blood is absent*.

Other bodies such as aloin, benzidin, the leuco-base of malachite green and phenol-phthalin can take the place of guaiac (see Blood in fæces, p. 305).

The function of hæmoglobin is to carry oxygen to the tissues. This power of taking up oxygen can easily be demonstrated by shaking up venous blood with air (see under Spectroscopy). The oxygen carrying capacity of blood can be ascertained as follows:—

**EXPERIMENT XIII.** In the bottle of a Dupré apparatus (see Fig. 257) take 20 c.c. of oxygenated blood and 30 c.c. dilute ammonia solution (1 am. : 500 water). In the small tube take 5 c.c. of fresh saturated potassium ferricyanide solution. Adjust the water to the zero of the apparatus by means of the clip and then upset the ferricyanide into the blood. Shake well. Readjust the level of the water and read how much oxygen has been given off.

## CHAPTER XII

### THE SPECTROSCOPIC EXAMINATION OF HÆMOGLOBIN AND ITS DERIVATIVES

A spectroscope consists essentially of a screen, in which there is a small *slit*, through which light from any desired source can pass, a *prism*, and a series of lenses forming the *telescope*, through which the observer looks.

For qualitative work the small direct vision spectroscope (Fig. 179) is serviceable. When the position of the bands, however, is required, one of the larger compound forms is necessary.

**Adjustment of the Spectroscope.**—It is necessary to have an exact focus of the image of the slit. In the *small direct vision spectroscope* this may be obtained by directing the instrument towards a white cloud, and moving the eye-piece till the various Fraunhofer lines are clearly defined, or, in absence of daylight, obtaining a clear image of the upper and lower edges of the slit, i.e. of the upper and lower edges of the spectrum. The slit should not be too widely open. If the source of light include a sodium flame, a clear image of the D-line will be obtained when the slit is in focus.

1. **The Visible Spectrum of Oxyhæmoglobin.**—Take some defibrinated blood which has been thoroughly shaken with air, and dilute it with about ten times its volume of water. Place some of this behind the slit of the spectroscope, preferably in a flat-sided vessel about 1 cm. thick, but a test tube will answer fairly well. It will be noticed that the whole of the spectrum is blocked out except a portion of the red end.

Dilute this solution carefully. At a certain stage some of the green will be evident (see Spectrum 3 in Chart), there being a wide



FIG. 179.—Small direct vision spectroscope.

absorption band between the red and green. On diluting still further, this wide absorption band will resolve itself into two bands (Spectrum 2). These two bands are both on the blue side of the D-line, and their centres correspond to  $\lambda$  579 and  $\lambda$  543.8. Note carefully the position of these centres on the scale and the width of the bands. Observe also the limits of the visible spectrum at the red and blue ends.

On diluting still further it may be possible to cause the band on the blue side to disappear, whilst the band on the red side is still just visible (Spectrum 1).

2. **The Visible Spectrum of Hæmoglobin (reduced Hæmoglobin).**—If some diluted defibrinated blood be left standing undisturbed for twenty-four hours, the oxyhæmoglobin will lose its oxygen. This result may be arrived at more rapidly by treating some diluted defibrinated blood which shows fairly wide oxyhæmoglobin bands with a reducing reagent, such as ammonium sulphide or Stokes' reducing fluid.<sup>1</sup> If ammonium sulphide be used, the mixture should be warmed. It will now be noticed that the blood loses its bright scarlet appearance and becomes more purple in tint. Examine this by the spectroscope, and it will be found that the

<sup>1</sup> Two grms. of ferrous sulphate are dissolved with 3 grms. tartaric acid in 100 c.c. of water. Ammonia is added till the solution is alkaline. Stokes' fluid must be freshly prepared.



FIG. 180.—Absorption-spectra.

1, Oxyhæmoglobin (very weak solution); 2, Oxyhæmoglobin (weak solution); 3, Oxyhæmoglobin (strong solution); 4, Hæmoglobin (reduced hæmoglobin); 5, Carbon-monoxide hæmoglobin; 6, Acid hæmatin; 7, Acid hæmatin (etheral extract); 8, Alkaline hæmatin; 9, Hæmochromogen (reduced hæmatin); 10, Hæmatoporphyrin (acid solution); 11, Hæmatoporphyrin (alkaline solution).

two bands of oxyhæmoglobin have disappeared, and are replaced by one band, the centre of which is between the two bands of oxyhæmoglobin. The band is a broad one, shading off more gradually on the red side, and the darkest part corresponds in wave-length to  $\lambda$  550 (Spectrum 4 in Chart).

**3. The Visible Spectrum of Carbon-Monoxide Hæmoglobin.**—If a stream of carbon monoxide, or even of coal-gas, be passed through some diluted defibrinated blood, the scarlet tint is changed to a carmine or cherry colour. The oxygen is replaced by carbon monoxide. Examined spectroscopically the blood shows two bands differing from those of oxyhæmoglobin in being slightly shifted towards the blue end. The two bands have centres corresponding in wave-length to  $\lambda$  575 and  $\lambda$  540 approximately (Spectrum 5).

The proportion of red and blue unabsorbed at the ends of the spectrum is different in oxyhæmoglobin and CO-hæmoglobin, there being more blue unabsorbed in CO-hæmoglobin than in the former. Hence, comparing dilute solutions of similar strength in test tubes of the same diameter, the CO-hæmoglobin has a distinct bluish tinge, contrasting markedly with the yellowish-red of the oxyhæmoglobin. This difference of end-absorption can be best shown as follows: Take a fairly dilute solution of oxyhæmoglobin showing the two characteristic bands clearly, but not strong enough to produce any intermediate shading. Note as carefully as possible where the red and blue are first visible. Pass a stream of coal gas or carbon monoxide through the solution by means of a fine nozzle for two or three minutes. Note the change in colour produced, and again examine the spectrum. It will now be found that rather more of the blue is visible, whilst the red is unaltered or slightly more absorbed.

An important difference between oxyhæmoglobin and CO-hæmoglobin is seen in the effect of reducing reagents. If CO-hæmoglobin be treated with Stokes' fluid or ammonium sulphide, it is unchanged.

**4. The Visible Spectrum of Methæmoglobin.**—To a solution of oxyhæmoglobin, in which the two bands are so wide as to partially overlap, add a few drops of a strong solution of potassium ferricyanide. The colour changes to a chocolate tint. If this be spectroscopically examined, a distinct band is seen on the red side of the D-line, the wave-length of its centre being about  $\lambda$  635 (Spectrum 12). On diluting the solution down, other bands may be seen—one just on the blue side of the D-line ( $\lambda$  581), another still further towards the blue ( $\lambda$  540), and a fourth may be made out on the bluish-green ( $\lambda$  500) (Spectra 13 and 14). The two middle bands are probably not due to any traces of oxyhæmoglobin, but are characteristic of methæmoglobin.

If such a solution of methæmoglobin be treated with ammonium sulphide, a transient spectrum of oxyhæmoglobin may be seen, succeeded by a permanent spectrum of reduced hæmoglobin.



If the solution of methæmoglobin be rendered alkaline with ammonia, the colour changes to a more distinct red, and the absorption band in the red disappears and is replaced by a band immediately on the red side of the D-line (Spectrum 15 in Chart).

By the action of nitric oxide on oxyhæmoglobin, a product is formed called **nitric oxide hæmoglobin**. This is characterised by two bands, which are between the D and E lines; the band on the red side is somewhat nearer the red end than the corresponding band of oxyhæmoglobin (Spectrum 16).

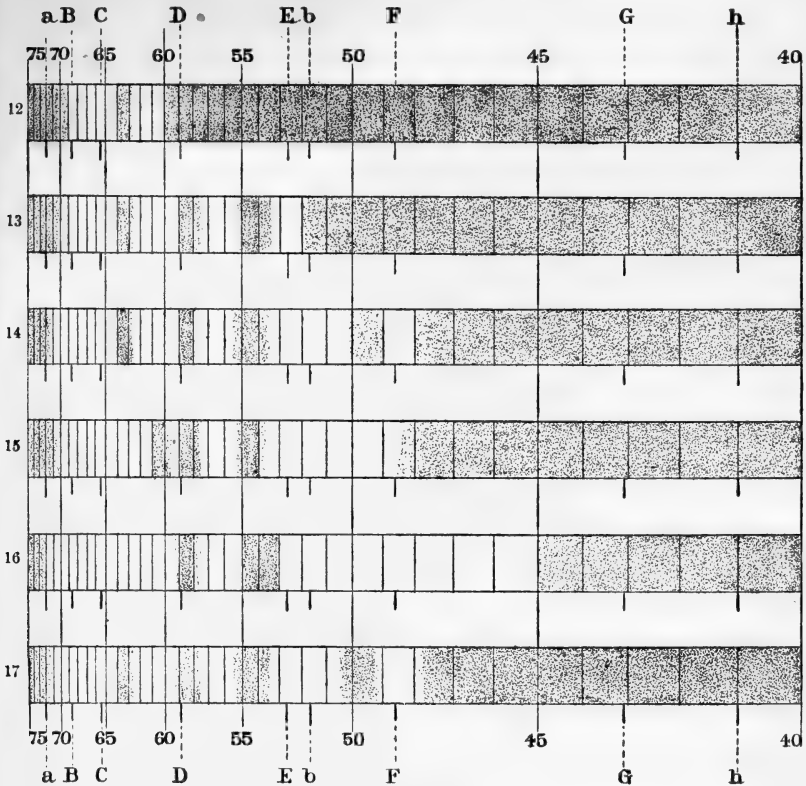


FIG. 181.—Absorption-spectra.

12, Methæmoglobin (strong solution); 13 and 14, Methæmoglobin (weak solutions); 15, Methæmoglobin (alkaline solution); 16, Nitric oxide hæmoglobin; 17, Mixture of methæmoglobin and nitric oxide hæmoglobin.

If oxyhæmoglobin be treated with a nitrite, as sodium nitrite or amyl nitrite,<sup>1</sup> there is formed a certain amount of methæmoglobin and a certain amount of nitric oxide hæmoglobin. The combination of the two gives a spectrum very similar to simple methæmoglobin (Spectrum 17 in Chart).

<sup>1</sup> Care must be taken to avoid excess of amyl nitrite, or so-called photo-methæmoglobin, characterised by one broad band between D and E, will be formed.

If the product of the action of nitrites be treated with ammonium sulphide, the spectrum passes through a transient oxyhæmoglobin stage, succeeded by reduced hæmoglobin, and later becomes nitric oxide hæmoglobin.

**5. The Visible Spectrum of Acid-hæmatin.**—If some diluted defibrinated blood be treated with a little glacial acetic acid and gently warmed, it will assume a dark brown colour. If it be diluted sufficiently, and examined spectroscopically, it will present a spectrum characterised by one band on the red, the wave-length corresponding approximately to  $\lambda$  645. The blue end of the spectrum will be very largely absorbed (Spectrum 6 in Chart).

There is frequently a considerable amount of general absorption in the acid-hæmatin prepared as above, and the band referred to may only be made clear by filtering the solution. More satisfactory results are obtained by extracting the colouring matter with ether, or treating with chloroform and excess of acetic acid, as follows:—

(a) Take defibrinated blood, and add about half its volume of glacial acetic acid and about an equal quantity of ether. Shake at once. The ether will extract the colouring matter, and, on examining the same spectroscopically, three distinct bands will be seen—one on the red similar to that already described, but apparently shifted nearer the D line ( $\lambda$  640), one on the green ( $\lambda$  550), another on the green but nearer the blue ( $\lambda$  515). A very indistinct band may be seen on the yellow ( $\lambda$  590) (Spectrum 7 in Chart).

(b) Take defibrinated blood, warm and add half its volume of glacial acetic acid. Cool and add half the volume of chloroform, and more acetic acid if any precipitate appears. The solution will become clear and give a spectrum similar to that shown in the ethereal extract.

**6. The Visible Spectrum of Alkaline Hæmatin.**—Take some diluted defibrinated blood, and add a few drops of strong caustic soda, and warm. The colour will change to a greenish-brown tint, and when the solution is examined spectroscopically, it will show a single band on the orange (wave-length,  $\lambda$  600). A more satisfactory method of preparing the alkaline hæmatin is to form a paste of potassium carbonate and defibrinated blood; dry it over a water-bath and extract with alcohol, when a reddish-brown solution is obtained which shows the distinguishing absorption band clearly (Spectrum 8 in Chart).

**7. The Visible Spectrum of Hæmochromogen (reduced Alkaline Hæmatin).**—If a watery solution of alkaline hæmatin be warmed with a few drops of ammonium sulphide, the brownish colour is replaced by a more marked red. If the solution be examined spectroscopically, the one band of alkaline hæmatin is found to be replaced by two bands on the green, the wave-lengths of their centres being approximately  $\lambda$  557 and  $\lambda$  525 (Spectrum 9).

**8. The Visible Spectrum of Hæmatoporphyrin.**—Take some strong sulphuric acid (10 c.c.) in a test tube and add a few drops of blood, and shake up the mixture. A purple colour will result, due to the decomposition of the hæmoglobin and the formation of the iron-free pigment, hæmatoporphyrin. This examined spectroscopically will, in the above acid solution, show two bands, the centres being approximately  $\lambda$  605 and  $\lambda$  565 (Spectrum 10 in Chart).

If a large excess of water is added to the above a precipitate is thrown down. If this be dissolved in a little caustic soda, a solution of hæmatoporphyrin in an alkaline medium is obtained, which shows a four-banded spectrum when examined, the positions of the bands being  $\lambda$  630,  $\lambda$  580,  $\lambda$  550, and  $\lambda$  520 approximately (Spectrum II in Chart).

Hæmatoporphyrin may be regarded as iron-free hæmatin, and identical in composition with bilirubin.

Solutions of hæmatoporphyrin exhibit a red fluorescence. This pigment must be regarded as normally present in small quantities in urine.

## CHAPTER XIII

### URINE

In studying the chemistry of the urine, we must ascertain, firstly, the nature of its various constituents and of their precursors in the blood and tissues; secondly, the total amount of those excretory products which contain the nitrogen of the decomposed proteins; and thirdly, we must look for unusual products, indicating improper composition of the blood or organic disease of the urinary tract.

We must remember that the quantity and the composition of the urine vary considerably within the limits of health, and in order to form reliable conclusions we must collect the total urine for a period of twenty-four hours. Even with a fair sample thus obtained, we must consider the intake and loss of water; copious drinking will increase the quantity and lower the specific gravity of the urine; on the other hand, profuse sweating or diarrhœa will have the opposite effect. The nature of the diet in relation to the reaction of the urine and the quantity of urea must also be considered.

#### General Characters of Urine

**Quantity.**—A healthy man of average weight (65–70 kg.) and height, and living on an ordinary mixed diet, excretes about 1,500 c.c. per twenty-four hours. If we wish to ascertain whether any one of its constituents is being excreted in normal amount, a knowledge of the total daily excretion of urine is indispensable, a mere determination of the percentage in an isolated sample being of *very slight* value. For accurate work the method employed is to collect the total urine for the twenty-four hours in a suitable vessel, and then to remove from this a measured sample for analysis.<sup>1</sup> The amount of urine is increased by the imbibition of large quantities

<sup>1</sup> In doing this, the bladder is emptied at some chosen hour (best in the morning), and this urine thrown away; all urine passed subsequently to this is collected in a sterile flask or bottle containing a few c.c. of chloroform, and at the same hour next day the bladder is again emptied and the urine added to the twenty-four hour specimen. When the observation is being conducted on the lower animals, it is usually necessary to employ the catheter.

of liquid and by certain drugs called diuretics ; it is diminished by excessive sweating or diarrhœa, and by failure of the heart's action.

**Specific Gravity.**—This is determined by a special form of hydrometer—a urinometer—graduated so that the zero mark—1,000—corresponds to distilled water (Fig. 182).

**EXPERIMENT I.** Fill a urine testing glass with urine cooled to room temperature, place the urinometer in it, and read off the graduation which is on a level with the surface of the urine. Be careful that the urinometer does not stick to the sides of the vessel.



FIG. 182.—The urinometer.

The average density varies between 1,015 and 1,025, but a highly concentrated urine, e.g. after severe sweating, may reach 1,035, or a very dilute one, e.g. after huge potations, 1,002, and still be healthy. A specific gravity over 1,030, however, usually indicates the presence of sugar or the existence of high fever, and one much below 1,010 should raise suspicions of renal trouble.

**Reaction.**—Healthy urine usually reacts acid to litmus. This acidity is due to sodium dihydrogen phosphate,  $\text{NaH}_2\text{PO}_4$ , not to free acid.

**EXPERIMENT II.** Test the reaction of urine with blue litmus paper and congo red paper. The litmus is turned red, but the congo red is not altered, as it is not affected by the acid salts of any but the strongest acids (see Digestion, p. 228).

The alkaline phosphate,  $\text{Na}_2\text{HPO}_4$ , may be present in urine. It is detected by the addition of calcium chloride to the urine, when a precipitate of calcium phosphate forms if the alkaline phosphate is present, but not if the acid phosphate alone is present. The amount of alkaline phosphate may be sufficient to cause the urine to have an amphoteric reaction, turning red litmus blue and blue litmus red, or even to have a definite alkaline reaction. This is often the case during the stage of digestion, when hydrochloric acid is being poured into the stomach, as the removal of hydrochloric acid from the blood leaves an excess of bases.

Besides the alkaline phosphate, alkaline bicarbonates may be present in urine, causing an effervescence on the addition of acid. This is the case when salts of oxidisable acids (e.g. citric, tartaric, etc.) are being taken by the mouth, and when the diet is an exclusively vegetable one. Lastly, an alkaline reaction may be due to ammonia, which is produced by bacterial hydrolysis of urea (see

Urea, p. 255). For this reason stale urine always reacts alkaline. If the alkaline reaction of freshly passed urine is due to ammonia, decomposition must be taking place in the bladder.

A very simple method of determining the distribution of the phosphate between the acid and alkaline salts has been introduced by Leathes. In a solution of sodium phosphate, if all the phosphate is acid phosphate, methyl orange turns pink: if any of it is dibasic this indicator is yellow; when it is all dibasic, and not before, phenolphthalein turns pink. To determine the nature of the phosphates present in the urine two 10 c.c. samples of urine well diluted with distilled water are titrated, the one with  $\frac{N}{10}$   $\text{H}_2\text{SO}_4$ , using methyl orange as indicator, and the other with  $\frac{N}{10}$   $\text{NaOH}$ , using phenolphthalein as indicator. The first titration gives the amount of dibasic phosphate present and the second the amount of acid phosphate. The ratio of the amounts of acid and alkali used in the two titrations gives the ratio in which the two phosphates are present. As the phosphates are the principal constituents of the urine which determine its reaction, the reaction of the urine may be expressed in the same way as that of a pure phosphate solution, in acidity or alkalinity per cent.

**Colour.**—The colour of urine varies in health from pale amber or straw to deep red brown.

(a) Nearly colourless due to dilution or diminution of pigments, as in polyuria from any cause, diabetes insipidus, certain diseases of the kidney.

(b) Dark yellow or brown red due to an increase of pigment or increase of concentration of urine as in acute febrile diseases or after profuse sweating.

(c) Red or reddish due to the presence of hæmoglobin or pigments used in colouring foodstuffs.

(d) Brown or brown black due to the presence of hæmatin, methæmoglobin, melanin or after carbolic acid poisoning.

(e) Brown yellow to red brown due to the presence of various colouring matters of vegetable origin, as after the administration of santonin, chrysophanic acid, senna, rhubarb, etc. Note the addition of  $\text{NaOH}$  turns urine which contains many of these vegetable dyes, red.

(f) Greenish yellow or yellow brown due to the presence of bile pigments, as in various hepatic diseases.

As regards the actual pigments, apart from those of definitely pathological origin or derived from substances administered to the subject, the principal pigment would seem to be *urochrome*. After the removal of this pigment from the urine the colour is largely lost. It is a sulphur containing pigment and has an acid reaction. It shows no absorption bands, but it cuts off the violet end of the spectrum. It does not fluoresce with zinc salts. *Urobilin*, mostly in the form of a colourless chromogen, is also present in very small amount in normal urine. In many pathological conditions urobilin is present in large amount. The chromogen is readily converted into urobilin by oxidation. *Uroerythrin* is also present in traces. (See p. 297.)

**Indican Test.**—To about 5 c.c. of urine in a test tube add an equal volume of concentrated hydrochloric acid and 3 c.c. of chloroform, then add drop by drop a dilute (freshly prepared) solution of bleaching powder. Shake the tube after the addition of each drop of the hypochlorite solution. The pigment, as liberated, is taken up by the chloroform. Care must be taken not to add too much of the bleaching powder, as the indigo blue formed can be further oxidised to colourless compounds.

In place of the bleaching powder, hydrochloric acid containing a small amount of ferric chloride (0.2 to 0.4 per cent.) may be used. Or it may be replaced by ammonium persulphate, say, 1 drop of a 10 per cent. solution.

## CHAPTER XIV

URINE (*Continued*)**Total Nitrogen and Urea**

The amount of total nitrogen excreted by the average man living on an average mixed diet is about 15 grams per day. As the amount of nitrogen excreted is dependent mainly on the nature of the diet, it is obvious that no hard and fast figure can be laid down for a mixed diet. And, further, unless a sample of a mixed twenty-four hours collection is taken the values obtained are for the most part absolutely worthless. Even a sample from the twenty-four hours urine is of little value unless the nature of the diet consumed during the period of collection is known. If a reliable sample of urine is required, the subject must be put on a simple fixed diet for at least two days before the twenty-four hour sample is collected.

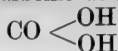
The chief nitrogenous substances present in the urine which collectively account for the bulk of the total nitrogen (T.N.) are urea, uric acid, ammonia and creatinine. Many other nitrogen containing substances are present in the urine, but as a rule they only occur in traces. Not only does the amount of total nitrogen excreted depend on the nature of the diet, but, as Folin has shown, the distribution of the component substances varies with the diet taken. Folin's table demonstrates this fact very clearly.

	N. rich diet.		N. poor diet.	
	Quantity.	% of T.N.	Quantity.	% of T.N.
Volume of Urine . . . .	1170 c.c.		385 c.c.	
Total Nitrogen . . . .	16.8 grm.		3.60 grm.	
Urea Nitrogen . . . .	14.7 "	87.5	2.20 "	61.7
Ammonia Nitrogen . . . .	0.49 "	3.0	0.42 "	11.3
Uric Acid . . . . .	0.18 "	1.1	0.09 "	2.5
Creatinine Nitrogen . . . .	0.58 "	3.6	0.60 "	17.2
Undetermined Nitrogen . .	0.85 "	4.9	0.27 "	7.3

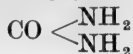
## Urea

Urea is commonly regarded as the diamide of carbonic acid,

Carbonic acid.



Urea.



but there is now a certain amount of evidence to show that in reality

it is isocarbamide with a formula  $\text{HN} : \text{C} \begin{cases} \text{NH}_2 \\ \text{O} \end{cases}$

In common with other acid amides, urea has weak basic properties, forming unstable salts with nitric and oxalic acids.

EXPERIMENT I. To some urine, which has been evaporated to small bulk on a water-bath, add some pure, colourless (not fuming) nitric acid, and cool the mixture by holding the test tube under the

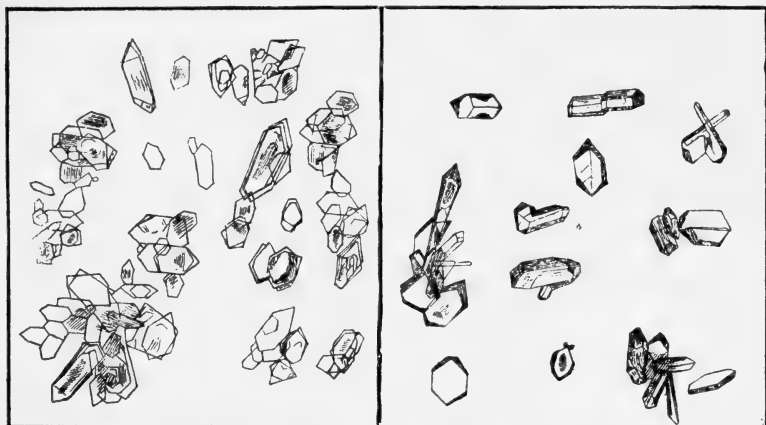


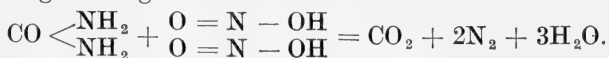
FIG. 183.—Urea nitrate.

FIG. 184.—Urea oxalate.

tap. Crystals of urea nitrate separate out. Examine these with the microscope, and note that they are either rhombic tables or six-sided plates, which overlap each other like the tiles of a roof (see Fig. 183).

EXPERIMENT II. Repeat experiment with a saturated alcoholic solution of oxalic acid, and note that the crystals are not unlike those of the nitrate, being elongated plates with bevelled pointed ends (Fig. 184).

Urea is decomposed by nitrous acid— $\text{HNO}_2$ —carbonic acid gas and nitrogen being evolved :



EXPERIMENT III. Add some fuming nitric acid (i.e. containing nitrous acid) to urine, and note the effervescence which results. That one of the gases evolved is carbon dioxide can be proved by





**Estimation of Urea.**—Many methods have been introduced for the estimation of urea. As an approximate estimation the hypobromite method is fairly good and has the considerable merit of being rapidly carried out. If accurate work is required on the whole the urease method will prove the most serviceable (see page 280).

**Hypobromite Method.**—As stated this method only gives, as usually carried out, an approximation, as urea yields variable amounts of nitrogen unless the strictest of conditions are adhered to, and further certain other urinary constituents also yield a certain amount of nitrogen. With all its limitations, however, the method has a certain value.

From the consideration of the formula representing the changes which take place (p. 256) it will be noted that when urea is decomposed by the hypobromite two gases, carbonic dioxide and nitrogen, are liberated. As a strongly alkaline solution of hypobromite is always used the free alkali combines with the carbonic acid at once so that nitrogen alone is measured.

Theoretically 1 grm. urea gives off 372 c.c. nitrogen at 0° and 760 mm., but in practice only about 354 c.c. are given off.

There are various forms of apparatus used for collecting the liberated nitrogen. That of Dupré (Fig. 185) consists of an inverted burette (*a*) placed in a cylinder of water, and to the neck of which is connected a T-piece (*f*). With the side tube of this the generating bottle is connected by india-rubber tubing, and the other tube is closed with a piece of tubing and a clip. To make the estimation, 25 c.c. of the freshly prepared alkaline solution of sodium hypobromite<sup>1</sup> are placed in the generating bottle (*o*) and 5 c.c. urine in a small tube, which is then carefully placed in the generating bottle without allowing the two fluids to mix. The cork of the generating bottle is then inserted, and the meniscus of the water both inside and outside the burette brought to the same level at the zero mark, the

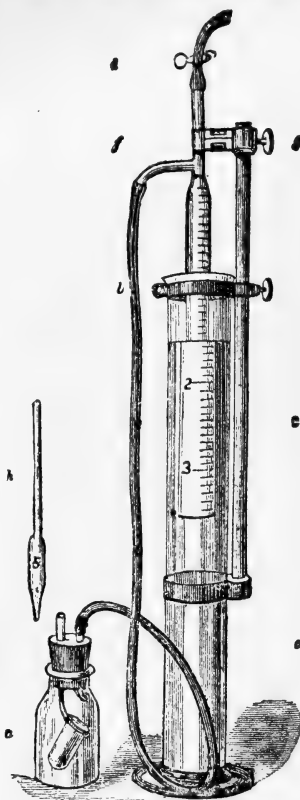


FIG. 185.—Dupré's urea apparatus.

<sup>1</sup> Add 25 c.c. bromine to a well-cooled solution of caustic soda made by dissolving 100 grms. NaOH in 250 c.c. water. If only a small amount is required addition of 2 c.c. bromine to 23 c.c. 40 per cent. NaOH suffices.

clip on the T-piece being open meanwhile, and water being added to, or removed from, the outer vessel if necessary. The clip is now applied, and the burette raised to ascertain that no leakage exists. The two menisci are then readjusted, and the contents in the generating bottle mixed. The evolved N displaces the water in the burette. After the reaction is complete, the generating flask is immersed in a basin of water, so as to bring the temperature of the gas contained in it to the same as that of the gas in the burette. After waiting two minutes the two menisci are again brought to the same level, and the number of c.c. of N read off. Do not hold or touch any part of the glass of the apparatus with the bare hand.

Calculation of Result.—If no correction for temperature and pressure be made it is very simple.

42·5 c.c. nitrogen were obtained from 5 c.c. urine tested and 35·4 c.c. are liberated from 0·1 grm. urea; therefore 42·5 c.c. equals approximately 0·12 grm. urea, i.e. in 5 c.c. urine there is approximately 0·12 grm. urea, and therefore in 1,500 c.c.,  $0·12 \times 300 = 36$  grms.

If correction for temperature and pressure be made this formula is used :

$$v = \frac{v' \times 273 \times (b - b')}{(273 + t) \times 760}$$

$v'$  = volume of gas evolved,  $b$  = barometric pressure,  $b'$  = tension of watery vapour at  $t$  = temperature at time of estimation.

For rapid clinical purposes quite satisfactory results may be obtained by using the small Doremus ureometer.

## CHAPTER XV

### URINE (*Continued*)

#### Purines, Creatinine and Ammonia

**Purines.**—There are always present in varying amount products of the decomposition of nucleo-protein, mainly in the form of uric acid and to a lesser extent as other purine bases. The amount of these substances present is largely influenced by the nature of the diet. After a meal rich in meat, more particularly one which has contained material like sweetbread, the output of purines is high, but they are also present although in diminished amount on a purine-free diet. It is possible that a certain limited amount of the purines may be of synthetic origin, i.e. not derived from the breakdown of nucleo-protein. In man uric acid may be regarded as the principal end product of the catabolism of nucleo-protein. The substances grouped under the heading of purine bases are the two amino purines, Adenine and Guanine and the two oxypurines,

Hypoxanthine and Xanthine. Uric acid is not present as such in the urine, but is combined with bases, like sodium, to form urates.

Two types of salts are formed: (a) the primary salts, the so-called mono or acid urates which are formed when uric acid in an aqueous solution acts as a monobasic acid, and (b) the secondary salts, the so-called di or neutral urates, which can only exist in the presence of excess of alkali. The group of salts which were formerly called quadri-urates is probably a mixture of uric acid with a primary salt. In urine then we have only to deal with primary salts.

### Preparation and Reactions of Uric Acid

**EXPERIMENT I.** To 100 c.c. urine add 5 c.c. HCl (conc.), and allow the mixture to stand overnight. It will then be found that a dark-brown sediment, like cayenne pepper, has settled down, and probably also that a brown scum has formed on the surface. Filter and examine the sediment under the microscope. It consists of large dark-brown clumps of crystals, whetstone or barrel-shaped (Fig. 186). These are crystals of uric acid admixed with pigment. They can be purified by solution in 5 per cent. KOH and reprecipitation by HCl. Preserve the crystals for further use.

**EXPERIMENT II.** Pure crystals can be obtained from the solid urine of a snake or bird. This urine is dissolved, with the aid of heat, in 5 per cent. caustic potash cooled, filtered and acidified with HCl. Pure uric acid separates out.

From these two experiments we learn that uric acid exists in urine as a salt. If this salt be decomposed by a mineral acid the liberated uric acid, being very insoluble, is precipitated.

The following are the most important reactions of uric acid.

**EXPERIMENT III.** *The Murexide Test.*—Place some uric acid on a piece of glazed porcelain, add a few drops of dilute nitric acid, evaporate *slowly* to dryness over a small flame. A yellow residue is obtained. Add after cooling a drop of ammonia, a crimson colour results, which is changed to purple by adding caustic soda. If overheated, the residue will turn crimson without the addition of ammonia. This test cannot be applied directly to urine as urine yields a red pigment on heating with nitric acid.

*Phosphotungstic Acid Test.*—Dissolve a few crystals of uric acid in about 2 c.c. very dilute sodium hydrate, then add 1 c.c. of Folin's uric acid reagent (see p. 283) and 10 c.c. of a saturated sodium carbonate solution. An intense blue colour develops. This test cannot be applied direct to urine as certain other substances, such as the phenols, present also give the same blue coloration.

**EXPERIMENT IV.** Uric acid has the power of reducing metallic oxides in alkaline solution. This may be demonstrated by the following tests. Some uric acid is dissolved in weak sodium carbonate solution, which is then poured on to a piece of filter paper moistened with a solution of  $\text{AgNO}_3$ . A black stain of reduced silver results. This is called Schiff's reaction. Uric acid can also exercise its reducing powers on cupric salts in alkaline solution. By applying Trommer's test, or one of its modifications, to an alkaline solution of uric acid, it will be noticed that reduction ensues. The reduction precipitate

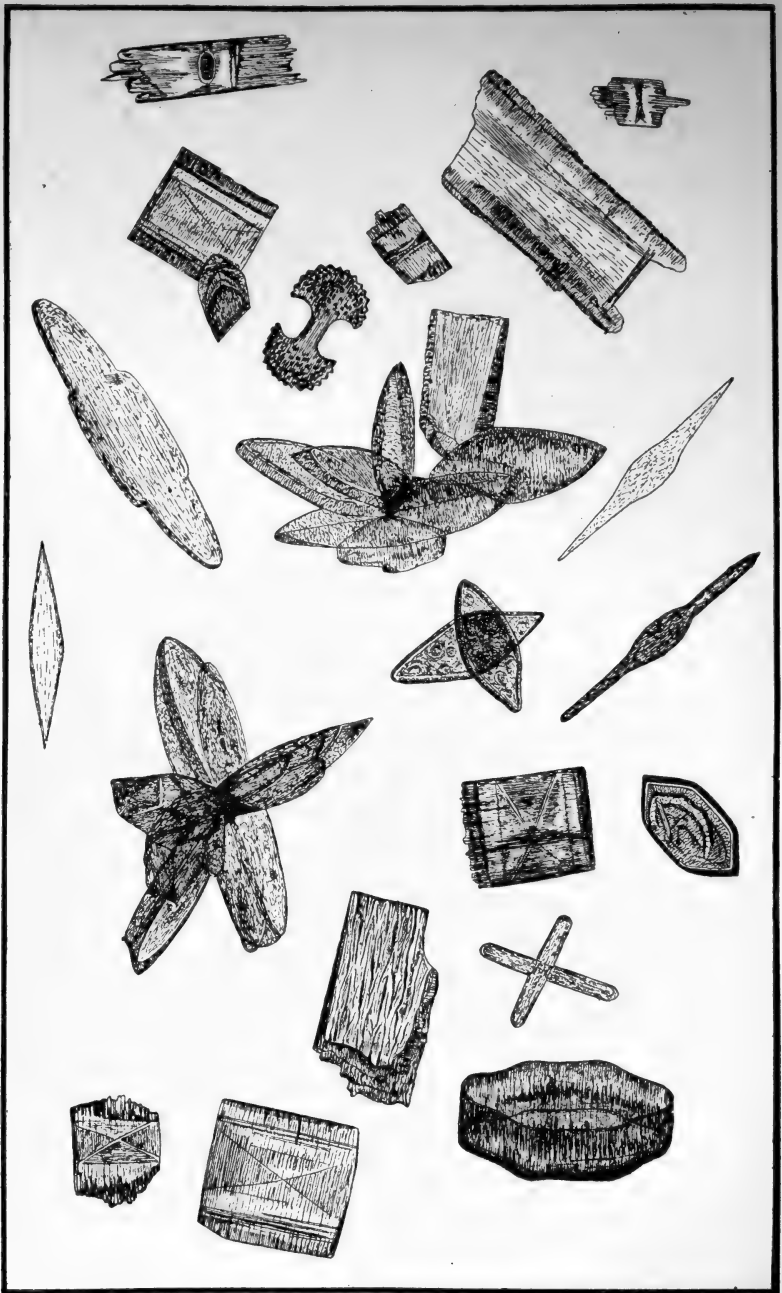


FIG. 186.—Crystals of uric acid.

is, however, of a dull brown colour instead of being yellowish red, as it usually is. This is because a certain amount of the cuprous oxide unites with some of the uric acid to form a brown compound.

**Hippuric Acid.**—This substance is found in marked amount in the urine of herbivora, but is only present in small amount in the urine of man on an ordinary mixed diet. It is chemically benzoyl-glycine and is formed from aromatic substances present in the food, particularly vegetable food-stuffs, being oxidised to benzoic acid which is then excreted combined with glycine.

**Creatinine.**—Urine always contains creatinine, the output on a creatinine-free diet being very constant for a given individual. Creatine is not normally present in the urine of the adult although it is normally present in the urine of young children and is frequently present in the urine of women at menstruation and in pregnancy. It can be made to appear readily by starvation and it is excreted in certain pathological conditions.

**Tests for Creatinine.**—**EXPERIMENT I. Jaffe's Test.**—Add to 5 c.c. urine a few drops of a saturated solution of picric acid and then NaOH until alkaline. A red brown colour is produced due to the formation of picramic acid. Do a check observation on 5 c.c. water.

**EXPERIMENT II. Weyl's Test.**—Add to 5 c.c. urine a few drops of a dilute solution of sodium nitro-prusside, then on the addition of dilute caustic soda solution a ruby red colour develops which quickly changes to yellow. If this solution be acidified with acetic acid and then boiled a greenish blue colour results and eventually on standing a precipitate of Prussian blue is formed.

Acetone gives the same colour with the nitro-prusside and soda, but (1) it does not turn yellow on standing, and (2) it gives a reddish purple colour on the addition of acetic acid.

**Simple Estimation of Creatinine (Burns).**—*Apparatus.*—Haldane's Hæmoglobinometer tubes. Standard tube contains the Folin standard solution of bichromate (see p. 285).

*Method.*—Pipette 10 c.c. of urine into a 250 c.c. measuring cylinder, add 5 c.c. 10 per cent. NaOH and 15 c.c. saturated solution of picric acid. Mix and allow to stand for exactly six minutes. Dilute with water to 250 c.c. Fill empty hæmoglobinometer tube up to 50 mark with the mixture, add water drop by drop until the colour of the mixture and the standard match. (It is better, as in the estimation of hæmoglobin, to note the point at which the colour is just too strong and the point at which it is just too pale and take the mean of these.) The reading gives the amount of creatinine present in milligrams per 100 c.c. of urine.

**Ammonia.**—The amount of ammonia in urine varies indirectly with the amount of urea, due to the fact that ammonia is the chief precursor of urea. If for any reason, such as the presence of acids in the body, ammonia is required for neutralisation, there will be a rise in the output of ammonia in the urine and a fall in the urea output. In certain pathological cases where acidosis is a marked feature the ammonia nitrogen may form a large proportion of the total urinary nitrogen.

The contrary condition is also true, namely, when there is an excess of alkali ingested, either in the food or otherwise, ammonia may practically disappear from the urine. This diminution in the output of ammonia is marked when, for instance, vegetable food-stuffs predominate in the diet.

**Estimation of Total Acidity and Ammonia in Urine.**—**EXPERIMENT.** Weigh out roughly 15 grms. powdered potassium oxalate (neutral to phenolphthalein), place in a flask, and add from a pipette 25 c.c. urine and an equal volume of water. Add about 10 drops 1 per cent. alcoholic phenolphthalein. Mix and allow to stand for about a minute. Now run in  $\frac{N}{10}$  caustic soda from a burette until the contents of the flask assume a slight pink tint. Read the burette. Measure into a small beaker 5 c.c. formalin (40 per cent. formaldehyde) and roughly 5 c.c. water, and add a few drops of phenolphthalein solution. Run in  $\frac{N}{10}$  caustic soda till a slight pink colour is attained. Add this mixture to the flask containing the neutralised urine. The pink colour disappears. Run in  $\frac{N}{10}$  caustic soda until the colour returns, and take the reading.

The first reading of the burette gives the total acidity of 25 c.c. urine in terms of  $\frac{N}{10}$  soda. Potassium oxalate is added to precipitate the calcium in the urine as calcium oxalate, as the formation of calcium phosphate would otherwise interfere with the end-point.

On the addition of neutral formaldehyde the ammonia in the urine combines with the formaldehyde, forming a neutral compound, urotropine, thus liberating the acid which it previously neutralised. The second titration, therefore, determines the amount of ammonia present in terms of  $\frac{N}{10}$  soda. To calculate the amount of nitrogen in grammes present as ammonia in the volume of urine taken multiply the reading of this titration in c.c. by 0.0014.

This method of determining ammonia is of sufficient accuracy for clinical purposes. The amount of ammonia is always higher by this method than by the more accurate methods which follow. This is due to the fact that formalin combines with amino acids, which are normally present in urine in minute traces, and thus renders them acid to phenolphthalein. This source of error is small, unless amino acids are present in excessive amount, as in cystinuria. The difference between the result of this method and that of one of the methods which follow affords a measure of the amount of amino acids present in the urine (see p. 286).

## CHAPTER XVI

URINE (*Continued*)**Inorganic Constituents of Urine. Deposits**

**Chlorides.**—The amount of chloride excreted varies largely with the amount present in the diet. Variation also takes place in certain pathological conditions.

**EXPERIMENT I.** Add to urine a few drops of nitric acid and then silver nitrate solution. A white precipitate of silver chloride forms which is soluble in ammonia. The nitric acid prevents the precipitation of other silver salts such as phosphate.

**Estimation of Chloride.**—There are two methods which are commonly employed, Mohr's and Volhard's. The latter is the more accurate of the two, but the former, which gives excellent results although slightly too high because other substances like purines combine with the silver before chromic acid does, is the more rapid.

**Mohr's Method.**—Take 10 c.c. of the urine, dilute with six or seven times its volume of water, add 5 drops of a cold saturated solution of potassium chromate. Then run in from a burette standard silver nitrate solution (1 c.c. = 0.01 gm. NaCl), stirring constantly until a permanent faint red colour of silver chromate appears.

c.c.  $\text{AgNO}_3$  sol. used  $\times 0.01 =$  grms. NaCl in 10 c.c.

Sometimes a correction is used on account of the too high value referred to, viz., the deduction of 1 c.c. from the actual number of c.c.  $\text{AgNO}_3$  used in the titration. (See p. 288.)

**Phosphates.**—These are found in the urine as :—

(i) Earthy phosphates, i.e. combined with calcium and magnesium.

(a) Render the urine alkaline by means of caustic soda. Earthy phosphates are precipitated. (b) Render alkaline with ammonia, allow to stand, then examine by means of microscope the precipitate of triple phosphate (feathery phosphates).

(ii) Alkaline phosphates, i.e. combined with sodium and potassium. Precipitate the earthy phosphates with NaOH, filter off the precipitate, acidify filtrate with acetic acid, then add uranium acetate solution and warm. A precipitate of uranium phosphate forms.

**EXPERIMENT II.** Boil some solution of ammonium molybdate in nitric acid in a test tube and add drop by drop boiling urine acidified with nitric acid. A yellow precipitate indicates the presence of phosphate.

**Estimation of Phosphates.**—Place 25 to 50 c.c. urine in a porcelain basin with 5 c.c. acid sodium acetate solution<sup>1</sup> and heat it until

<sup>1</sup> Acid sodium acetate solution; 100 grms. sodium acetate, dissolved in water, 100 c.c. glacial acetic acid made up to 1,000 c.c.

it is gently boiling, then run in from a burette a standard  $\left(\frac{N}{10}\right)$  solution of uranium acetate (1 c.c. of  $\frac{N}{10}$  uran. acet.<sup>1</sup> = 0.00355 grm.  $P_2O_5$ ) until a drop of the boiling mixture placed on a drop of potassium ferrocyanide on a white glazed tile gives an immediate brown colour due to the formation of uranium ferrocyanide, thus indicating that there is now an excess of uranium present. In taking out drops for testing care must be taken to see that the glass rod employed does not come in contact with the mouth of the burette and get contaminated with uranium solution, otherwise erroneous results will be obtained :

c.c.  $\frac{N}{10}$  uran. acetate used  $\times$  0.00355 = grms.  $P_2O_5$  in 25 (or 50) c.c.

Cochineal added to the mixture in basin may be used as indicator in place of the ferrocyanide. End reaction is change of red colour to green. (See also p. 288.)

**Sulphates.**—Sulphur is excreted in three forms in the urine as (a) preformed (inorganic) sulphates, (b) ethereal sulphates, and (c) neutral sulphur. The bulk of the excretion is in the form of inorganic sulphate.

**EXPERIMENT III.** Add about 2 c.c. HCl to 10 c.c. urine in a test tube and then an excess of barium chloride solution. A white precipitate of barium sulphate forms. Filter off this precipitate and boil the clear filtrate. A further precipitation of barium sulphate should take place due to the decomposition of ethereal sulphates by the hydrochloric acid. (See also p. 289.)

**Metabolism.**—One of the results of Folin's investigations on metabolism has been to show the significance of determinations of sulphates in the urine. The total sulphur in the urine is, like the nitrogen, distributed among several substances, which are divided into three groups—the inorganic sulphates, the ethereal sulphates, and the neutral sulphur compounds. The inorganic sulphates are mainly those of sodium; the ethereal sulphates are compounds of phenol, cresol, scatoxyl, and indoxyl, with sulphuric acid and potassium (see p. 238), and the neutral sulphur compounds are organic compounds in which the sulphur is an integral part of the molecule. Cystin, when present, belongs to this group. When the relative amounts of  $SO_3$  excreted in the above-mentioned three forms are calculated as percentages of the total  $SO_3$  excretion, it is found that the inorganic sulphates on a protein-poor diet behave like urea-nitrogen, i.e. become less both in absolute amount and in relative percentage; that the neutral sulphur under the same conditions behaves like creatinin-nitrogen, i.e. remains constant in absolute amount, whereas the percentage rises, and that the ethereal sulphate excretion behaves like that of ammonia-nitrogen, i.e. becomes somewhat less in absolute amount, but that the percentage rises.

These facts are clearly shown in the following table, which is an extension of that on page 254.

---

<sup>1</sup>  $\frac{N}{10}$  uranium acetate, 21.3 grm. ur. acet. dissolved in water and made up to 1 litre.



	N.-rich diet.	N.-poor diet.
Volume of urine	1,170 c.c.	385 c.c.
Total nitrogen	16.8 gm.	3.60 gm.
Total SO <sub>3</sub>	3.64 gm.	0.76 gm.
Inorganic SO <sub>3</sub>	3.27 gm. = 90.0% of total S.	0.46 gm. = 60.5% of total S.
Ethereal SO <sub>3</sub>	0.19 gm. = 5.2% ,,	0.10 gm. = 13.2% ,,
Neutral SO <sub>3</sub>	0.18 gm. = 4.8% ,,	0.20 gm. = 26.3% ,,

The ethereal sulphates cannot, as has been supposed, derive their source entirely from the aromatic bodies formed in the intestine by micro-organismal growth. When this is excessive, or when there is obstruction in the small intestine so that an excessive amount of these aromatic bodies is absorbed, an increase no doubt occurs in the ethereal sulphate excretion, but this increase can be no reliable index of intestinal putrefaction, since the relative ethereal sulphate excretion becomes greater when the diet contains little or no protein. Practically the only source of sulphur intake by the food is in proteins. Sulphates are not taken unless for medicinal purposes, because of their disagreeable taste. The sulphur excretion by the urine is therefore a measure of protein catabolism in the organism.

### Urinary Deposits

Normal urine is quite clear when it is passed, but, on standing some time, a sediment usually separates out, and this varies somewhat under different conditions.

*Acid Urine from a healthy person may deposit the following :—*

1. **Urates** (see p. 259).—The sediment has a chalky appearance and is usually tinged reddish by uroerythrin. It disappears on warming the urine. Examined microscopically, it is generally amorphous, but may show a crystalline structure, usually as needles, or as balls with spines projecting from them (Fig. 187). It is composed mainly of sodium urate.

2. **Uric Acid**.—It appears as a cayenne pepper-like sediment, and has a definite crystalline appearance under the microscope (Fig. 186). The crystals may vary much in shape, but are always large and tinged a reddish colour. The most usual shapes for the crystals to assume are "sheaves," "whetstones," "rhombic tables," and sometimes "dumb-bells." The presence of the crystals does not necessarily indicate an increased excretion of uric acid, but depends on the concentration and acidity of the urine.

3. **Calcium Oxalate**.—This is usually a scanty deposit, adhering to irregularities on the surface of the glass of the urine jar, or forming a glistening layer on the top of the mucous deposit that settles at the bottom.

The crystals are insoluble in acetic acid. This reaction distinguishes them from phosphates or carbonates. They are also insoluble in ammonia, and are thus distinguished from urates.

Microscopically they are seen to be very small octahedra, often flattened along one axis, so that they appear like squares with diagonal lines (hence called "envelope" crystals, Fig. 190).

*Acid urine from a person suffering from disease, or during the administration of certain drugs, may deposit :—*

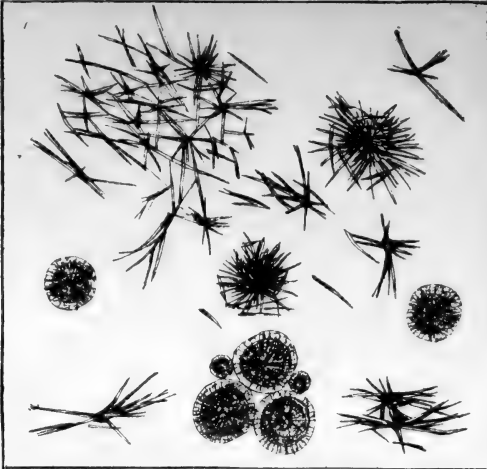


FIG. 187.—Sodium urate.  $\times 350$ .

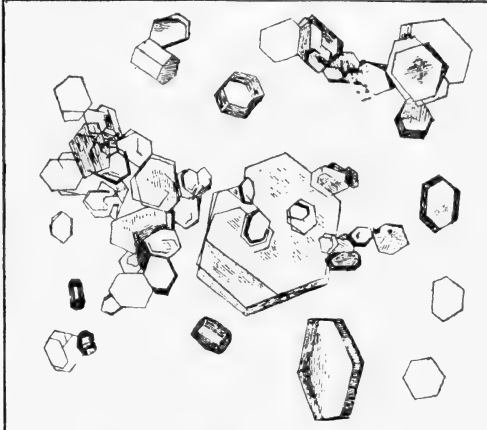


FIG. 188.—Cystin.  $\times 350$ .

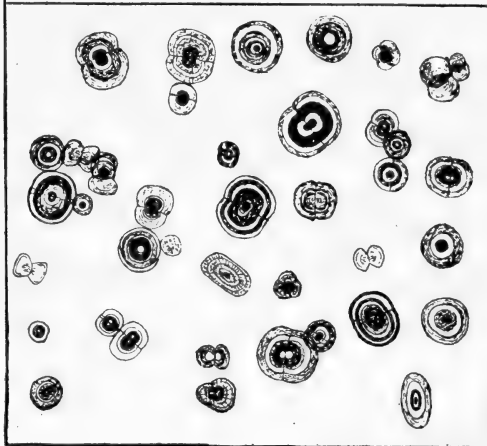


FIG. 189.—Calcium carbonate (from human urine).  $\times 400$ .

1. **Cystin.**—This forms a deposit somewhat like that of urates in appearance.

Microscopically, however, it shows a distinct crystalline structure consisting of hexagonal colourless plates or slabs (Fig. 188). When the crystals are present the condition is called cystinuria.

2. **Leucin and Tyrosin.**—Though very rarely, these two bodies sometimes occur in urine (e.g. in severe hepatic disease), where their appearance is similar to that in a pancreatic digest (see Fig. 234).

3. **Hippuric Acid.**—This may appear in urine during the admin-

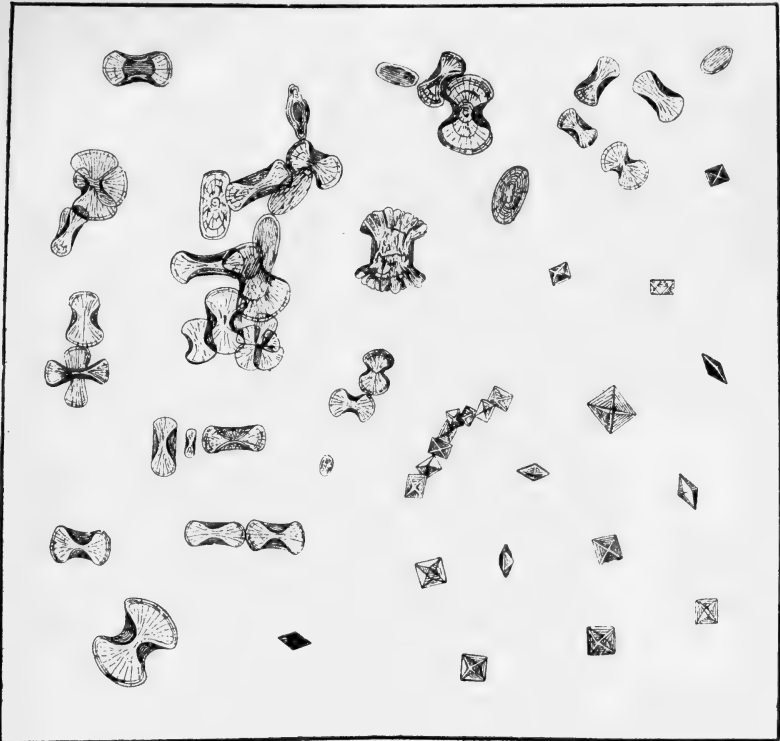


FIG. 190.—Calcium oxalate.  $\times 500$ .

istration of benzoic acid. It crystallises in four-sided prisms. It is quite common in the urine of herbivora.

*In Alkaline Urine the following may occur:—*

1. **Phosphates.**—Of these there are two kinds, viz., phosphate of calcium and ammonium-magnesium phosphate.

(a) **Phosphate of Calcium.**—The sediment is chalky and never pigmented; it clears up on adding a few drops of nitric acid; it is increased by boiling. Microscopically it is usually amorphous, but

may exist as long prismatic crystals arranged in star-shaped clusters, hence called *Stellar Phosphates* (Fig. 191). The crystalline form may also occur in faintly acid urines.

(b) Ammonium-magnesium Phosphate, Triple Phosphate.—When urine gets stale and ammonia develops in it, a white sediment and a white surface film form. Under the microscope these are seen to be

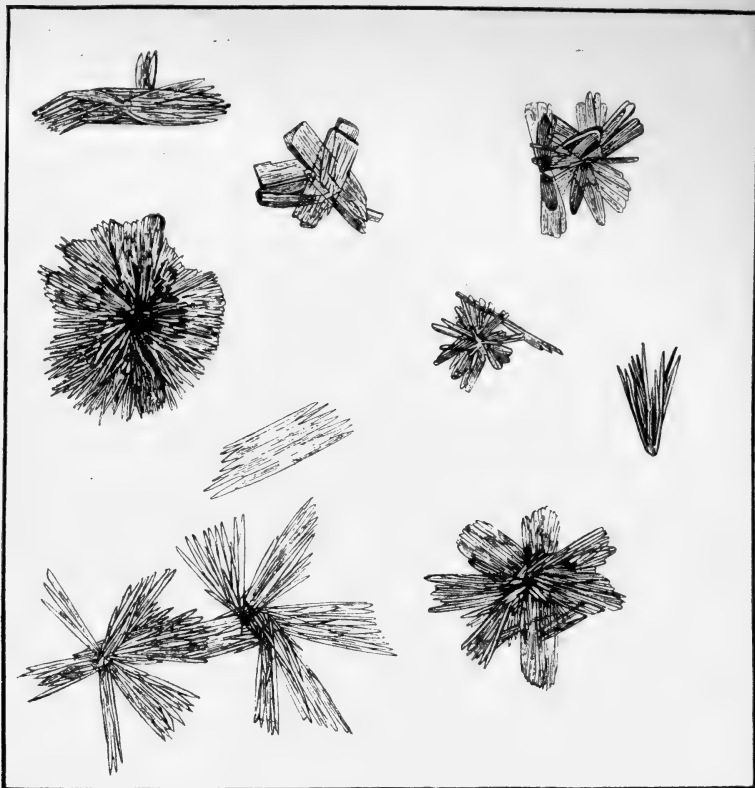


FIG. 191.—Stellar phosphate of calcium.  $\times 500$ .

made up of large clear crystals like "*knife rests*," or, if excess of ammonia be present, they may look like "*feathery stars*." This latter type can be easily obtained by adding ammonia to normal urine (Fig. 192).

2. **Urate of Ammonia.**—This looks like the urate of soda crystals, but is associated with crystals of phosphates, and occurs in an alkaline urine.

3. **Carbonates.**—In the urine of vegetarians these are not uncommon. The urine effervesces on adding acetic acid. Micro-

scopically the sediment is usually amorphous, but may exist as biscuit-shaped crystals or as dumb-bells (Fig. 189).

## CHAPTER XVII

## PATHOLOGICAL URINE

**Proteins in the Urine—Albuminuria.**—Traces of mucin or nucleo-protein may be added to the urine in its passage along the urinary tract, but otherwise healthy urine does not contain any protein. When the kidneys or urinary passages are diseased, however, a certain amount of the plasma proteins leaks into the urine, where they can be recognised by certain tests, the condition being called *Albuminuria*.

Also when proteins other than *serum* albumin and globulin gain access to the blood, they are at once excreted in the urine. It is on this account that albuminuria results after the consumption of a large number of raw eggs (egg flip) because the intestinal epithelium allows a certain amount of the unchanged protein to pass into the blood, where it is foreign (in being egg- and not serum-albumin), and is consequently excreted by the kidneys. In certain diseases of bone, a substance somewhat similar in its reactions to a proteose is added to the blood and is excreted by the urine (Bence Jones' proteosuria).

Although globulin may occur along with albumin in the urine, or even in some cases independent of it, it is of no practical importance to distinguish between them, so that the tests about to be described include both bodies.

The tests employed depend on certain of the reactions described under proteins. It is obvious that the colour reactions will not be applicable to the urine; those employed depend on the production of coagula. The most important of these are:—

**EXPERIMENT I. Heat Coagulation.**—Place some *clear* urine in a test tube, and boil. A white turbidity or coagulum indicates the presence of either albumin or phosphates (earthy phosphates are precipitated by boiling). To the boiling solution, whether it show a turbidity or not, add 3–4 drops of concentrated nitric acid. If due to phosphates, the turbidity will disappear, but will remain if due to protein. In nitric acid any acid- or alkali-albumin which the urine may contain is insoluble. Where there is doubt as to the occurrence of a haze, the test tube should be about three-quarters filled, and only the upper layer should be boiled, the test tube being meanwhile held low down. By holding it against a dark background the slightest haze becomes very evident by this method, on account of contrast with the unboiled layer beneath.

**EXPERIMENT II. Heller's Test.**—Place some clear urine in a test tube. Hold the test tube in a slanting position, and allow concentrated *pure* nitric acid to run *very slowly* down the side, so that it

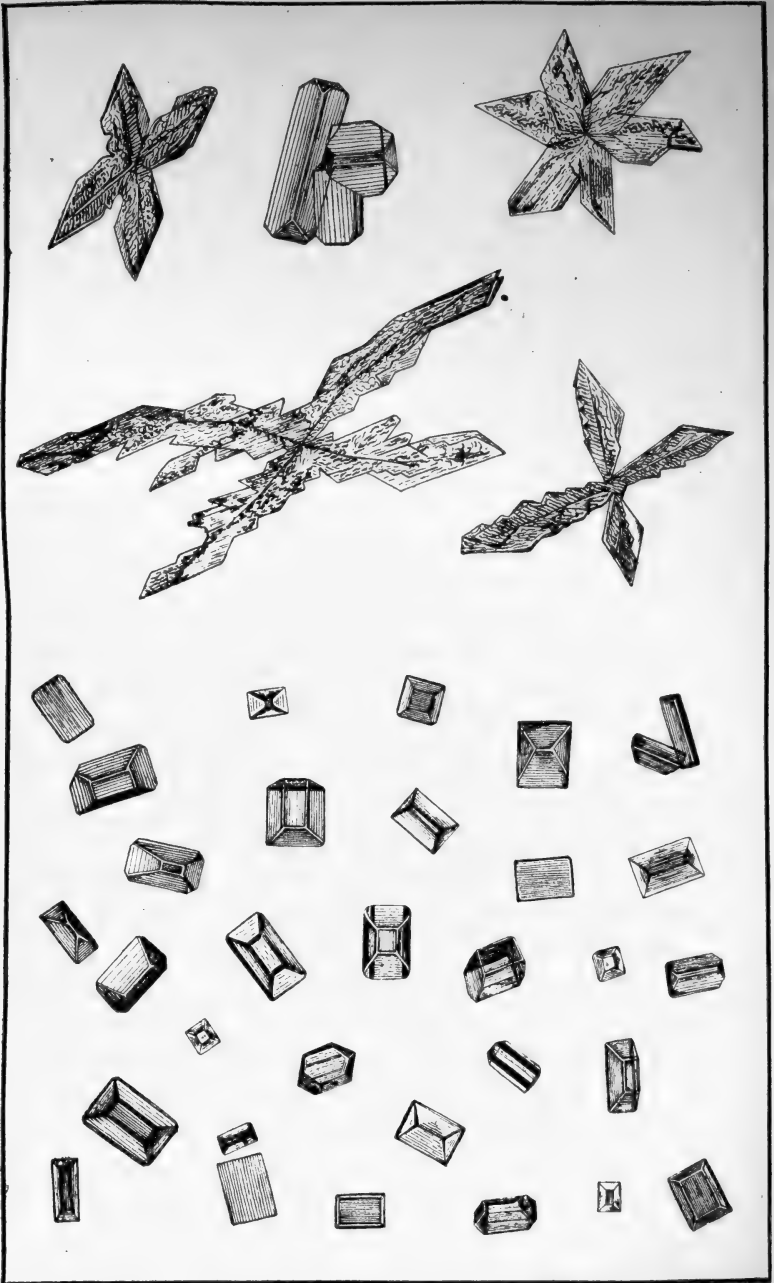


FIG. 192.—Triple phosphates.  $\times 400$ .

forms a layer underneath the urine. Where the two meet, a sharp white ring (of coagulated acid albumin) is formed. The test may also be done by placing the nitric acid first in the test tube, and covering this with the urine slowly delivered from a pipette. The ring does not disappear on warming. A similar ring may be obtained when proteoses are present, but in this case the ring clears up on gently warming the test tube, and reappears on cooling. In warming, very great care must be taken that no mixing of the two layers occurs. When mucin is present in excess a *diffuse haze* may be produced in the portion of urine next the acid. Certain resin acids which may appear in the urine after the administration of such drugs as copaiba also give a haze by Heller's test. Also when the urine is very concentrated, acid urates or urea nitrate crystals may develop and simulate the reaction. In these cases, the urine should be diluted with two or three times its bulk of water, and the test reapplied, when very little doubt will remain as to the reaction.

*Salicyl-Sulphonic Acid Test.*—This is perhaps the most delicate of all the tests.

EXPERIMENT III. Add to about 10 c.c. of urine a drop or two of a saturated solution of pure salicyl-sulphonic acid. A white precipitate results, which on boiling changes into a number of coagula.

This reaction occurs in a dilution of 1-230,000 albumin. The only other body with which this reagent produces a precipitate is proteose, in which case, however, the precipitate disappears on warming.

The reagent, if pure, keeps indefinitely. If impure, however, it turns red on keeping. It has the great advantage over nitric acid in being non-corrosive, and therefore easily carried about.

There are numerous other tests, but their application is superfluous if the above be properly applied.

**Proteoses** are detected by the precipitates produced by nitric and salicyl-sulphonic acids clearing up on heating the urine, and returning when it is cooled. The so-called "proteose" in Bence Jones' proteosuria is coagulated by moderate heat, but redissolves on boiling the urine. Proteose can best be separated from albumin by adding salicyl-sulphonic acid, boiling and filtering. The coagulated albumin remains on the filter paper, and the proteose is gradually precipitated in the filtrate as it cools.

**Quantitative Estimation of Albumin.**—For clinical purposes this is done by means of *Esbach's albuminometer* (Fig. 193). The determination is made by measuring the depth of the coagulum produced by adding to the urine Esbach's reagent (a mixture of 10 gms. picric acid and 20 gms. citric acid in 1,000 c.c. distilled water).

EXPERIMENT IV. Place clear urine, filtered if necessary, in an Esbach's tube up to the mark *U*. If the reaction be alkaline, render slightly acid by the addition of acetic acid; and if the specific gravity be above 1,008 dilute it with water till this density, or something

below it, is obtained.<sup>1</sup> Now add the reagent up to the mark *R*. Close the tube with a tightly-fitting cork and invert several times, so as to mix the fluids thoroughly. Allow to stand in an upright position for twenty-four hours, and then read off the graduation corresponding to the top of the precipitate. This gives the number of grammes of dried albumin per litre of urine used. If the urine has been diluted the necessary calculation must be made in order to obtain the percentage in the original urine.

For more accurate estimation of albumin, Scherer's method is employed.

**EXPERIMENT.** Place 50–100 c.c. urine (according to amount of albumin in it) in a large-sized evaporating dish, and, while stirring, bring to the boil. Carefully add a few drops of dilute acetic acid, and allow the coagulum to settle down. If the supernatant fluid is opalescent, add a little more acetic acid, and bring again to the boil. (It is very important to use as little acetic acid as possible, so that acid albumin may not be formed.) The coagulum must then be collected on a small ash-free filter paper which has been dried between watch-glasses at 120° C. After being collected on the filter, wash the coagulum with boiling water, followed by alcohol and ether, and dry it at 120° C. until the weight is constant. Since the coagulum contains considerable ash, the filter and coagulum must now be transferred to a weighed crucible, incinerated, and the weight of ash deducted from the weight of dried coagulum.



FIG. 193.—  
Esbach's  
albumino-  
meter.

**Mucus, Pus, and Casts in Urine.**—When the kidneys or urinary passages are diseased, besides protein there may be a considerable deposit of mucus in the urine. This body has the general properties and solubilities of mucin (see p. 201), but may consist largely of nucleo-protein. Casts also occur in the deposit from the urine. When these come from the urinary passages, they consist of groups of flattened epithelial cells. When they come from the kidney tubules, they are tubular and consist of polyhedral cells, showing various stages of degeneration. When the kidneys or urinary passages are infected by micro-organisms, pus cells occur in the urine and form

a deposit. Strong potash dissolves the pus, forming a viscid solution. Pus also gives a guaiac test as for blood, but much more slowly and not after boiling, which destroys the oxidases to which the test is due. The only certain test for pus, however, is to examine the urine or deposit with the microscope. The pus cells appear as colourless, spherical, highly refractive granular bodies, about 9  $\mu$  in diameter, the nuclei of which can be stained by adding dilute methylene blue. The urine is usually acid when the pus comes from the kidney, and alkaline when the pus comes from the bladder, due to the decomposition of urea and ammonium carbonate.

**Hæmoglobin in Urine.**—This may be due to bleeding from the kidneys or urinary passages, when it is called *hæmaturia*, or to

<sup>1</sup> These corrections should be made before the urine is measured into the Esbach's tube.



excretion of hæmoglobin or methæmoglobin from the blood plasma by the kidneys, called respectively *hæmoglobinuria* and *methæmoglobinuria*.

In any case the tests for hæmoglobin can be applied. The guaiac test, which is very sensitive, should be applied after boiling the urine to destroy oxidases. The spectroscopic examination is also very sensitive when an adequate depth of urine is employed (see p. 245).

**Hæmaturia** is distinguished by the smoky appearance of the urine and by examination of the urine, or deposit on centrifugalising, when red blood corpuscles are seen. The spectroscope nearly always shows the presence of oxyhæmoglobin. Blood from the kidney is mixed with the urine. That from the bladder is often present as a clot. If the red corpuscles have disintegrated, the urine will present the appearance of hæmoglobinuria. If the urine is stale, methæmoglobin may be present.

In **Hæmoglobinuria** and **Methæmoglobinuria** red blood corpuscles are not seen, and the urine is clear, not smoky. The two conditions are distinguished by the colour of the urine and by the spectroscope.

**EXPERIMENT V.** Test the urine supplied for blood and hæmoglobin.

**Bile in Urine.**—When the bile duct is blocked by a calculus, or its mucous membrane is swollen from catarrh, the bile, which accumulates in the bile channels, is reabsorbed into the blood-vessels and carried to the tissues, which become stained with bile pigment. Under these conditions the urine contains bile constituents, the most easily recognised of which are the bile pigments.

**EXPERIMENT VI.** Apply Gmelin's test (see p. 236) to the urine of a jaundiced patient. Where only a small quantity of bile pigment is present it is better to concentrate the pigment by proceeding as follows:—

Add calcium chloride solution to the urine, and then sodium carbonate solution, so as to form a precipitate of calcium carbonate and phosphate, which carries down the pigment; filter off the precipitate and dissolve it in as small a volume of hot dilute hydrochloric acid as possible; apply Gmelin's test to this solution.

Also apply Hay's sulphur test for bile salts (see p. 236).

**Sugars in the Urine.**—Although the presence of sugar in the urine is normally associated with the disease known as diabetes mellitus, it must not be forgotten that every case of glycosuria is not of necessity a case of diabetes. The relation of the blood sugar content (see p. 318) to the urinary condition is of prime importance. Further, there are always traces of sugar to be found in perfectly normal urine quite apart from the reduction which takes place with Fehling's solution, for example, due to the presence of creatinine, uric acid, urochrome, etc.

The other sugars which the urine may contain are **lactose** and **pentose**. The former of these is sometimes found in the urine of

nursing mothers, and the latter may appear in the urine when pentoses are given in the food.

**Tests for Dextrose in the Urine.**—The tests for dextrose, as described, can, with slight modifications, be applied to its detection in urine.

The most important of these are:—

**EXPERIMENT VII. Fehling's Test.**—Boil 5 c.c. of Fehling's solution in order to ascertain that the Rochelle salt which it contains has not decomposed into reducing bodies. If no reduction occur, add a drop of the suspected urine and boil again. If no result, go on adding small quantities, boiling between each addition, till 5 c.c. have been added.

Fehling's test is quite satisfactory, when sugar is present in considerable quantity. When the amount of reduction is small, however, it may be due to the presence of other substances than sugar. In such cases the following tests should be applied, as they are positive for sugars only.

**EXPERIMENT VIII. Boettger's Test.**—To 10 c.c. urine add 1 c.c. Nylander's reagent.<sup>1</sup> Heat for five minutes on the water bath. If sugar is present to the extent of at least 0.08 per cent., a black precipitate of bismuth forms.

Though not so delicate, the following tests are valuable, in that they indicate the nature of the sugar:—

**EXPERIMENT IX. 1. The Fermentation Test.**—Place some diabetic urine in a small beaker, and boil it on a sand bath for ten minutes, to expel any air it may contain. Cool the urine and test its reaction; if alkaline, render faintly acid with a weak solution of tartaric acid. (This precaution is necessary in order to prevent putrefaction, which would lead to the evolution of ammonia.)

Add a small piece (about the size of a split pea) of yeast, and stir it in the urine until a milky solution is obtained. Now transfer the fluid to a Doremus ureometer so that the upright limb is completely filled with fluid. Place this in an incubator, or in a warm place, as on the mantelpiece, overnight when it will be found that gas—carbon dioxide—has collected in the upper portion of the vertical limb.

Two control tubes—one with a weak solution of dextrose and yeast, the other with normal urine and yeast—should be arranged so as to prevent any fallacy due to inactive or impure yeast. If the amount of sugar present be very small, the fermentation test may give a negative result.

<sup>1</sup> Nylander's solution: dissolve 4 gms. Rochelle salt in 100 gms. of a caustic soda solution of 1.12 sp. gr.; add 2 gms. Bismuth subnitrate and heat on water bath until it is dissolved.



FIG. 194.—Ureometer.

Lactose and pentose do not give a positive result by this test.

2. *The Phenyl Hydrazine Test.*—The method of employing this is described on p. 207. The obtaining of characteristic dextrosazone crystals is positive evidence of the presence of dextrose; glycuronic acid (p. 278) also gives crystals, but less readily.

**Estimation of Dextrose in Urine.**—The polarimeter (see p. 212) may be employed for the estimation of dextrose in urine. The main objection to its use is that optically active bodies besides dextrose, e.g. glycuronic acid and oxybutyric acid, which are lævo-rotatory, occur in diabetic urine, and therefore to a certain extent vitiate the result. The other method is to determine the reducing power of the urine.

*Fehling's Method.*—The *standard solution* contains 34.64 gms. pure crystallised copper sulphate, 180 gms. Rochelle salt and 70 gms. caustic soda per litre. 10 c.c. of this solution are equivalent to 0.05 gm. dextrose.

The urine is diluted exactly 10 or 20 times according to the amount of sugar present and placed in a burette. 10 c.c. of the standard solution are measured with a pipette into a porcelain basin, diluted with 40 to 50 c.c. water and heated to boiling. The solution is kept just boiling, and the diluted urine run in carefully with stirring, until the blue colour of the solution has just disappeared. From the volume of diluted urine required in the titration the amount of dextrose in grammes present in 100 c.c. of the original urine is calculated. Several determinations must be made. A flask heated on a water-bath may be substituted for the basin in order to minimise the risk of oxidation of cuprous oxide.

Either of the two following methods may be used. They are both easy to carry out and both give excellent results. They are to be preferred to the Fehling method.

1. *Folin's Method.*—Reagents. (1) Copper sulphate solution containing 59 gms.  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and 2 c.c. of concentrated sulphuric acid per litre (to prevent precipitation of copper hydrate by traces of alkali from the glass bottle). (2) Saturated solution of sodium carbonate containing 14–20 per cent.  $\text{Na}_2\text{CO}_3$ . (3) Alkaline phosphate mixture. Mix thoroughly in a mortar 200 gms. crystallised disodium phosphate ( $\text{HNa}_2\text{PO}_4 \cdot 12\text{H}_2\text{O}$ ) and 50 gms. of sodium (or 60 gms. potassium) thiocyanate. To the semi-liquid paste which is formed add 100–110 gms. anhydrous sodium carbonate and mix until the compound forms a granular powder. Keeps indefinitely, but should be kept in stoppered bottles. Folin recommends that the small 5 c.c. burette used for holding the sugar solution should be provided with a fine accessory tip attached by rubber tubing to the burette so that the drops will be small and uniform.

Measure out approximately 5 gms. (not less than 4.5 gms. nor more than 5.5 gms.) of the phosphate mixture into a large (boiling) test tube containing 5 c.c. of the copper solution and 1 c.c. of the

carbonate solution. Heat gently about  $60^{\circ}$ , with shaking, until all the salts except for a few isolated particles of sodium carbonate have dissolved. A practically clear solution is usually obtained in less than one minute. Add a glass bead to prevent bumping when heating with sugar.

From the filled sugar (or urine) burette add about 0.5 c.c. to the warm clear copper solution, boil very gently—by moving the test tube to and fro through the flame—for two minutes *by the watch*. If all the copper is reduced, blue colour completely disappears, the urine contains over 5 per cent. sugar and the determination must be repeated with a more dilute solution. If, however, the contents of the test tube are but slightly reduced yielding only a small precipitate of cuprous sulphocyanate, a further amount of sugar solution may be added: boil gently for one minute. Repeat the additions of sugar and the boiling for one minute until complete reduction has taken place. Confirm the result by a repetition, the first addition of sugar solution or urine being only 3 or 4 drops less than the full amount required, boil for three minutes, then add the rest of the urine a drop at a time till complete reduction. Boil for one minute after each addition. The total boiling period for a correct titration must not be less than four nor more than seven minutes. But the preliminary titration may last for eight to nine minutes, and if the boiling process has been gentle the result will be then only about 1 per cent. too high.

Calculation.—2.5 divided by titration figure in c.c. = per cent. glucose present.

End point is very sharp with pure dextrose solutions, with urine the end point is the transition from a greenish to a yellow (pale) colour.

2. *Benedict's Method*.—Dissolve by the aid of heat in about 600 c.c. of water, 200 gms. sodium citrate, 100 gms. anhydrous sodium carbonate, and 125 gms. potassium thiocyanate, cool, filter, make up to about 800 c.c. with water. Pour into this solution very carefully and with constant stirring a solution (100 c.c.) containing exactly 18 gms. pure copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) prepared in a litre flask. Then pour the mixed solution back into the measuring flask (which contained the copper solution) without loss, add 5 c.c. of a 5 per cent. solution of potassium ferrocyanide (to prevent any precipitation of cuprous oxide) and make up the total volume to 1,000 c.c. with the rinsings of the beaker used to mix the two solutions.

Measure 25 c.c. of the reagent into a 150 c.c. flask or a porcelain basin; add about 4 gms. anhydrous sodium carbonate, heat to boiling and whilst boiling run in the sugar solution (or urine) until the last of the blue colour disappears. The addition of the sugar solution should be at such a rate that the boiling solution is kept nearly constant in volume during the operation. If the urine contains a large amount of sugar it should be diluted so that not less than 10 c.c. will be required to reduce the 25 c.c. of reagent employed.

Calculation.—5 divided by volume of sugar solution used = per cent. sugar.

Amount of sugar which reduces—

	10 c.c. Fehling.	25 c.c. Benedict.	5 c.c. Folin.
Glucose . . . .	0.05 gm.	0.05 gm.	0.025 gm.
Lævulose . . . .	0.052 „	0.052 „	0.025 „
Lactose . . . .	0.067 „	0.067 „	0.0404 „ (anhydrous)
Maltose . . . .	0.074 „	0.074 „	0.045 „ ( „ )

Normal human urine has an average reducing power equivalent to about 0.2 per cent. dextrose. Of this reducing power about 18 per cent. is due to dextrose, 8 per cent. to uric acid (see p. 259), and 25 per cent. to creatinine (see p. 261), the remaining 50 per cent. being probably due to urochrome. Furthermore, the colour of urine renders the end-point of the titration much more uncertain than when a watery solution of dextrose is employed.

Sometimes the urine contains *pentose* (*i*-arabinose). In such cases it reduces but does not ferment with yeast; it gives Tollen's test (p. 326).

The presence of *lævulose* is revealed by Seliwanoff's test (p. 210). It must not be forgotten that many urines give a definite red colour on heating with concentrated hydrochloric acid alone.

**The Acetone Bodies in Urine.**—These substances are:—

- (1)  $\beta$ -oxybutyric acid,  $\text{CH}_3 \cdot \text{CHOH} \cdot \text{CH}_2 \cdot \text{COOH}$ .
- (2) Aceto-acetic acid,  $\text{CH}_3 \cdot \text{CO} \cdot \text{CH}_2 \cdot \text{COOH}$ .
- (3) Acetone,  $\text{CH}_3 \cdot \text{CO} \cdot \text{CH}_3$ .

Aceto-acetic acid is an oxidation product of  $\beta$ -oxybutyric acid. Acetone is formed from aceto-acetic acid by the loss of carbon dioxide. A solution of aceto-acetic acid partially decomposes to acetone at ordinary temperatures. On boiling the decomposition becomes complete.

Acetone is present in minute traces in normal urine. All three bodies make their appearance in human urine when fat is being metabolised at an unusually rapid rate. They are present therefore in the urine of severe cases of diabetes, in the urine of starvation, and in the urine of many people when the carbohydrate of the diet is reduced below 70 gms. per diem. Under these conditions the amount of the acetone bodies is increased by exercise.

**EXPERIMENT X. Tests for Acetone. Legal's Test.**—Add to the urine in a test tube a few drops of a fresh solution of sodium nitroprusside and then caustic soda solution till definitely alkaline. A permanent red colour develops, which becomes deeper and assumes a purplish tint on acidifying with strong acetic acid. (Compare with test for creatinine.)

**Rothera's Test.**—Add a few drops of sodium nitroprusside solu-

tion, ammonia till alkaline, and saturate the liquid with ammonium sulphate crystals. A deep colour similar to that of permanganate develops and reaches its maximum in fifteen minutes. This test is more sensitive and distinctive than Legal's.

*Iodoform Test.*—Distil a few c.c. of the urine with a few drops of dilute sulphuric acid. To the distillate add a few drops of iodine in potassium iodide solution and caustic soda till the iodine colour disappears. Iodoform is precipitated, and is detected by the characteristic smell.

**EXPERIMENT XI.** *Test for Aceto-acetic Acid.*—To the urine add ferric chloride solution in excess of that required to precipitate the phosphate present. A deep red colour in the solution indicates the presence of aceto-acetic acid. (Carbolic and salicylic acids in the urine give a very similar colour.)

**Homogentisic Acid** is di-oxyphenyl-acetic acid  $C_6H_5$ ,  $\begin{matrix} \left\langle \begin{matrix} OH \\ OH \\ CH_2COOH. \end{matrix} \right. \end{matrix}$  It

reduces Fehling's solution. When present in the urine it causes the latter to become of a dark-brown colour on standing, or this change in colour may be hastened by adding some alkali. It can be easily separated from the urine by adding a solution of lead acetate, filtering off the precipitate of inorganic salts which at first forms and allowing the filtrate to stand, when large needle-shaped glancing crystals of the lead salt separate out. If these be collected and treated with sulphuretted hydrogen, so as to remove the lead, the acid is obtained in a pure state.

**Glycuronic Acid.**—Chemically this is dextrose in which the end— $CH_2OH$ —group has become oxidised to form  $COOH$ , or carboxyl. It has, accordingly, the formula  $COOH-(CH.OH)_4-CHO$ . It is an intermediate body in the metabolism of dextrose, and usually becomes further decomposed in the organism, to yield carbon dioxide and water. Sometimes, however, it unites with the aromatic bodies (phenol, scatol, etc.) absorbed from the intestine to form a salt. In this combination it takes the place of sulphuric acid (see p. 264). In very small amount, it seems to be always present in the urine, but under certain conditions (as after the administration of certain drugs) it becomes increased to such an extent as to impart to the urine a very considerable power of reducing metallic oxides in alkaline solution. When this is the case it is apt to be confused with dextrose. The only absolute test whereby it may be distinguished from dextrose is that it does not ferment with yeast. It gives the pentose reactions.

**Lactic Acid**, see p. 294.

# ADVANCED AND QUANTITATIVE METHODS

## CHAPTER XVIII

### QUANTITATIVE EXAMINATION OF URINE

**Estimation of Total Nitrogen by Kjeldahl Method.**—Pipette off 5 c.c. of the urine into a 300 c.c. combustion flask, add 10 to 15 c.c. N.-free  $\text{H}_2\text{SO}_4$  and a small piece of copper foil (2–3 mm. square). Heat this mixture in a fume chamber, carrying on the combustion until the solution is colourless, then allow to cool. Now transfer the cold colourless solution to a 1,000 c.c. distilling flask of hard glass, washing out the combustion flask thoroughly with distilled water until the total volume in the distilling flask is about 500 c.c. Add a drop or two of alizarin red solution (a 1 per cent. solution in water of sodium alizarin sulphate) and then almost neutralise the contents with strong (40 per cent.)

NaOH solution, cool. Measure 50 c.c. of  $\frac{N}{10} \text{H}_2\text{SO}_4$  into an Erlenmeyer or other suitable flask of about 500 c.c. size, add a few drops of alizarin red solution and place below the condenser, taking care that the connecting tube dips below the surface of the acid. (Indicator is added at this stage so that if too little acid has been taken to neutralise the  $\text{NH}_3$  distilled over early evidence may be obtained and more acid can be added.) Now make the contents of the distilling flask distinctly alkaline by a further addition of caustic soda, care being taken not to add a huge excess, and *immediately* connect with the condenser. The bunsen below the distilling flask is now lighted and the distillation continued until all the ammonia has distilled over: this requires about half an hour. The addition of a few pieces of porous earthenware or of granulated zinc to the distilling flask before it is finally made alkaline assists materially in the prevention of bumping. When the distillation is ended the distilling flask is removed and the condenser tube is well washed down with distilled water into the flask containing the  $\frac{N}{10} \text{H}_2\text{SO}_4$ .

When cool this acid is titrated with  $\frac{N}{10} \text{NaOH}$  and the difference between the amount of  $\frac{N}{10}$  alkali in c.c. required to neutralise completely the  $\frac{N}{10}$  acid remaining and the amount of  $\frac{N}{10}$  sulphuric acid

originally taken gives the amount of ammonia (or nitrogen) distilled over.

**EXAMPLE.** If 50 c.c.  $\frac{N}{10}$   $H_2SO_4$  be taken in the first instance and if at the final titration 25 c.c.  $\frac{N}{10}$   $NaOH$  are required, then

50 c.c. - 25 c.c. = 25 c.c.  $\frac{N}{10}$   $H_2SO_4$  neutralised by the  $NH_3$  evolved.

25 c.c.  $\frac{N}{10}$   $H_2SO_4$  = 25 c.c.  $\frac{N}{10}$   $NH_3$  = 25 c.c.  $\frac{N}{10}$   $N$

and as 1 c.c.  $\frac{N}{10}$   $N$  = 0.0014 gm.  $N$

then in 5 c.c. urine there is  $25 \times 0.0014 = 0.035$  gm.  $N$ , and therefore in 1,500 c.c. 10.5 gms.  $N$ .

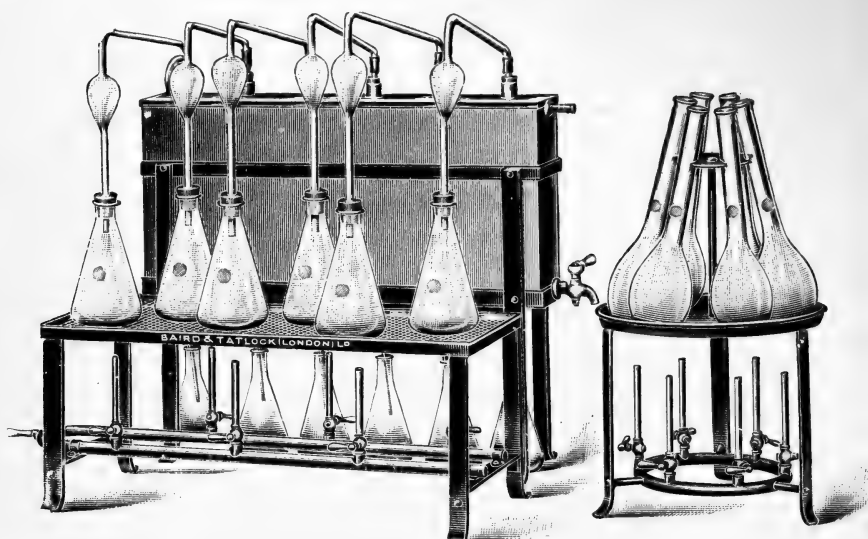


FIG. 195.

**Preparation of Urea from the Urine.**—Take about 50 c.c. urine in a porcelain basin and evaporate to dryness on the water bath. As soon as it is dry turn out the flame heating the bath, add about 10 c.c. acetone and allow the extraction to continue on the warm water bath for several minutes. Pour off the hot acetone extract, filtering through glass wool, into a large dry clock glass or glass evaporating basin. Urea crystallises out in long silky needles. It can be purified if desired by recrystallisation from alcohol. The yield obtained is about 1 gm. per 50 c.c. urine.

**Estimation of Urea. Urease Method.**—Soy beans contain a specific enzyme urease which rapidly and quantitatively decomposes urea at a low temperature into carbonic acid and ammonia.

**Method of Estimation.**—The following modification of the ordinary urease method, devised by Wishart, has been found to give reliable results.



The ordinary ammonia-absorption apparatus is modified as shown in the diagram. The constrictions in all three tubes should be placed at such a level that the capacity of the lower bulb is about three times the volume of the liquid within it. If this precaution be taken the use of toluol is unnecessary in most cases.

To complete the experiment in the times given the pump used should be capable of drawing air through the apparatus at a rate of not less than  $5\frac{1}{2}$  litres per minute. Water bath temp.  $40^{\circ}\text{C}$ .

Into tube A place 20 c.c. water, 5 c.c. urine, and 3–5 gms. soy bean

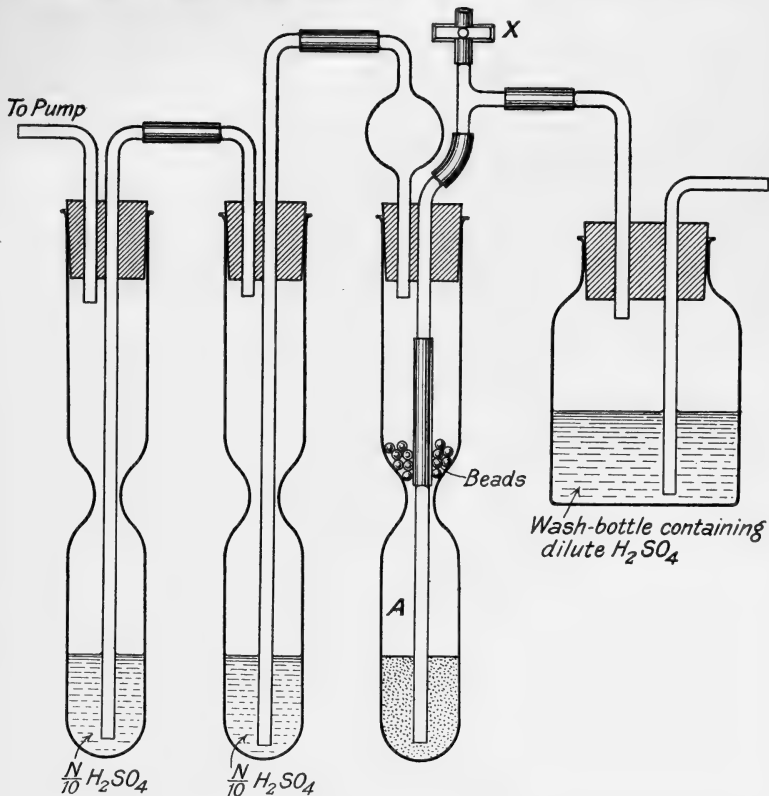


FIG. 196.

meal (varying amounts are required according to the condition of the bean; a slight excess will not vitiate the result). Mix the contents of the tube and insert the central tube and glass beads as shown, immerse bulb in water bath, then connect up to collecting tubes containing a known amount of decinormal acid. Start the pump and introduce about 2 c.c. of a saturated solution of sodium carbonate from a pipette by holding the nozzle of the pipette at X and opening the clip. Close the clip and aerate for forty minutes.

Calculation.—The amount of decinormal acid neutralised by the

liberated ammonia less the amount neutralised by the preformed ammonia in the urine (which must be determined by separate experiment) multiplied by 0.003 gives in grammes the amount of urea in the urine used. For ammonia determination, see p. 286.

A smaller apparatus with tubes of about 100 c.c. capacity, and using fiftieth normal acid, has been found useful for estimating small amounts of urea. The quantities for this apparatus are 5 c.c. of urea-containing fluid (content not more than 10 mgms. urea); 1 gm. soy bean meal; and  $\frac{1}{2}$  c.c. saturated sodium carbonate solution. Aeration with the small apparatus is complete in twenty minutes.

Since the time required for complete liberation of the ammonia depends on the velocity of the air-current and the amount of soy bean meal added, it is well to determine that all conditions are correct by doing one estimation on a solution containing a known amount of urea. The soy bean meal liberates a certain small amount of ammonia itself, so that, for accurate work, it is necessary to estimate this by doing a blank experiment using water in place of urea solution.

*Folin Method.*—Sometimes it is convenient and useful to use an acid hydrolysis method, such as that introduced by Folin, where the urine is heated to 150°–160° C. in the presence of an acid.

Weigh 20 gms. of crystallised magnesium chloride into an Erlenmeyer flask (250–300 c.c. capacity), then add 5 c.c. urine, 6 c.c. concentrated HCl, a small piece of hard paraffin wax or a few c.c. of liquid paraffin and finally a drop or two of an aqueous solution (1 per cent.) of alizarin red. A special safety tube (a U-shaped condenser) is then inserted in the mouth of the flask and then the mixture is boiled, best on a hot plate, until distinct “bumping” commences. The heat is then lowered until each drop as it falls back (about three times a minute) into the flask causes a slight bump. Heating is continued for at least two hours. If the indicator shows any appearance of a red colour during the hydrolysis a few drops of the acid distillate in the condenser must be returned to the flask by tilting the condenser. When the hydrolysis is complete the contents of the Erlenmeyer are transferred to a distilling flask, as in the Kjeldahl method, and after rendering *just* alkaline with caustic soda the ammonia is distilled off. Deduct the value for ammonia which must also be determined (see p. 286).

$$1 \text{ c.c. } \frac{N}{10} \text{ H}_2\text{SO}_4 = .003 \text{ gm. urea} = .0014 \text{ gm. urea nitrogen.}$$

This method serves excellently for the indirect estimation of *allantoin* as both urea and allantoin if present are hydrolysed under the above conditions, whereas by the urease method urea alone is attacked. Therefore if the amount of nitrogen obtained by the urease method is deducted from the yield by the acid hydrolysis method the difference may be assumed to be due to allantoin.

**Estimation of Uric Acid.** *Hopkins-Folin Method.*—Reagent necessary. (1) Uranium solution; 500 gms. ammonium sulphate, 5 gms. uranium acetate and 60 c.c. 10 per cent. acetic acid in 650 c.c. water.

(2)  $\frac{N}{20}$  potassium permanganate. (3) 10 per cent. ammonium sulphate solution.

100 c.c. urine is measured into a tall, narrow cylinder and 25 c.c. of the uranium solution is added in order to precipitate mucoid substances. The mixture is allowed to stand without stirring for about half an hour.

The uranium precipitate has then settled and the clear supernatant fluid may be removed by decantation (filtration if necessary). 100 c.c. (= 80 c.c. urine) of this fluid is measured into a beaker, 5 c.c. of strong ammonia added, and the mixture allowed to stand overnight. The precipitate is then filtered off, washed with 10 per cent. ammonium sulphate solution until chloride free. The filter paper is removed from the funnel, unfolded, and the precipitate washed back into the beaker with water, the filter paper and enough water to make about 100 c.c. added. The precipitate is now dissolved by the addition of 15 c.c. concentrated  $H_2SO_4$  and *at once*, whilst warm, titrated with  $\frac{N}{20}$  potassium permanganate solution. The end point is the first appearance of a permanent pink colour. (Note this colour will disappear within about half a minute.)

1 c.c.  $\frac{N}{20}$  potassium permanganate = 3.75 mgms. uric acid.

A correction of + 2.4 mgms., due to the solubility of ammonium urate, is added to the result.

*Folin and Wu Colorimetric Method.*—Transfer 1–3 c.c. of urine, according to concentration, to a centrifuge tube and add water to a volume of about 6 c.c. Add 5 c.c. of a silver lactate solution (5 per cent. silver lactate and 5 per cent. lactic acid) and stir with a fine glass rod. Rinse off the rod with a few drops of water. Centrifuge hard for two to three minutes. Add a drop of silver lactate solution to make sure an excess is present; if a precipitate (of AgCl) is formed add 2 c.c. more of the silver solution and centrifuge again; if no precipitate forms pour off the liquid as completely as possible.

To the precipitate in the centrifuge tube add, from a burette, as it is very poisonous, 4 c.c. of a 5 per cent. sodium cyanide solution. Stir the mixture until the precipitate is completely dissolved. Rinse the stirring rod, collecting the rinsings in a 100 c.c. graduated flask; pour the contents of the centrifuge tube into the same flask and rinse the tube three times with about 5 c.c. water. Add 5 c.c. of a 10 per cent. sodium sulphite solution (to balance the sulphite in the standard uric acid solution) and dilute to a volume of about 40 c.c. In another 100 c.c. flask place 5 c.c. of a standard uric acid sulphite solution containing 0.5 mg. of uric acid; add 4 c.c. of cyanide solution and about 35 c.c. of water. Then add 20 c.c. of 20 per cent. sodium carbonate solution to each flask and finally add *with shaking* 2 c.c. of the uric acid phosphotungstic reagent.<sup>1</sup> Allow to stand three to five minutes, fill to the mark and mix.

Set the standard uric acid solution at 20 mm. in both colorimeter cups and adjust the colorimeter until the two fields are exactly alike. Then empty one of the cups, rinse it out with the urine sample to be tested, fill and compare against the standard uric acid solution.

Calculation: Since the standard is only 0.5 mg., 10 divided by the reading of the unknown (in mm.) gives the amount of uric acid (in mg.) in the volume of urine taken.

Be careful to pour the discarded blue uric acid cyanide mixtures

<sup>1</sup> Preparation of uric acid reagent. Into a litre flask introduce 750 c.c. water, 100 gms. sodium tungstate and 80 c.c. phosphoric acid (85 per cent.  $H_3PO_4$ ). Put a small funnel with a watch glass in the mouth of the flask to act as a condenser, then boil gently for two hours. Cool and dilute to 1,000 c.c.

directly into the drain pipes of the sinks to avoid all risk of generation of HCN.

**Standard uric acid solution.** Dissolve in a 500 c.c. flask exactly 1 gm. of uric acid in 150 c.c. of water by the aid of 0.5 gm. lithium carbonate. Dilute to 500 c.c. and mix. Transfer 50 c.c. to a litre flask: add 500 c.c. of 20 per cent. sodium sulphite solution; dilute to 1,000 c.c. and mix. Transfer to small bottles (200 c.c.) and stopper tightly. In unopened bottles this solution keeps almost indefinitely, in opened bottles it remains good for two to three months.

**Estimation of the Total Purine Bodies.** *Modified Camerer's Method.*—*Principle.*—Ammoniacal silver nitrate, in the presence of neutral salts, or, better, of magnesium mixture, combines with all the purine bodies to form an insoluble salt of definite composition. The nitrogen in this can be estimated by Kjeldahl's method, and the result expressed as total purine nitrogen. This method is exceedingly useful in studying the metabolism of purine bodies.

*Solutions Necessary.*—1. Magnesia mixture. This consists of crystallised magnesium chloride 110 gms., ammonium chloride 110 gms., and .880 ammonia 284 c.c., made up with water to 1,000 c.c.

2. Ammoniacal silver nitrate. Dissolve 26 grs. silver nitrate in about 300 c.c. water, add ammonia to this until the precipitate of silver oxide, which first forms, redissolves. Dilute the solution to 1,000 c.c.

3. Kjeldahl's apparatus and solutions (see p. 279).

*Determination.*—240 c.c. protein-free urine are mixed with 30 c.c. magnesia mixture, and 30 c.c. of a 20 per cent. ammonia solution. This process is best done in a measuring cylinder. After the precipitate has settled, which it does in a few minutes, it is filtered through a dry folded filter and two portions of the filtrate are taken amounting to 125 c.c. each. Each of these corresponds to 100 c.c. of the original urine. They are both treated in exactly the same way, and should yield similar results. Each is mixed with 10 c.c. ammoniacal silver nitrate, and the mixture, after the precipitate has settled somewhat, filtered through an ash-free filter paper (of 10 cm. diameter). The last traces of the precipitate are removed from the beaker by means of weak ammonia water. The next stage consists in washing the precipitate with distilled water at 60° until it is free from ammonia, as the presence of this would vitiate the determination of the nitrogen. In order to do this, the precipitate should be allowed to stand exposed to the air overnight so that it may become partially dried, in which state the washing with water is much easier than when the precipitate is moist, for then it forms a gummy mass. The washing must be continued until the washings no longer react alkaline to litmus. In order to remove the last traces of ammonia, the filter paper, with the precipitate on it, is carefully removed to a Kjeldahl's combustion flask; about 50 c.c. of water and a little (0.5 gm.) magnesium oxide are added. The mixture is then boiled, whereon the magnesia expels the ammonia. The boiling is continued until only about 10 c.c. of fluid remain, and then 20 c.c. sulphuric acid, etc., are added, and the nitrogen determined in the usual way.

**Estimation of Creatinine.** *Folin's Method.*—The urine must be free from aceto-acetic acid and hydrogen sulphide, and must contain not more than traces of acetone. Measure 10 c.c. urine with a pipette into a 500 c.c. graduated flask. Add 15 c.c. saturated aqueous picric acid solution (about 1.2 per cent.) and 5 c.c. 10 per cent. caustic soda solution. Mix and allow to stand for five minutes. Fill up the

flask to the 500 c.c. mark, and mix well. By means of a Dubosq or other suitable colorimeter determine the depth of liquid required to give in daylight an intensity of colour exactly equal to that given by a depth of 8 mm. of a solution containing 24.55 gms. pure potassium bichromate per litre. (If pure creatinine zinc chloride is available a better standard is prepared by dissolving 1.6106 gms. of the salt in water in a litre flask, add 100 c.c.  $\frac{N}{1}$  HCl and make up to 1,000 c.c. ; 1 c.c. contains 1 mg. creatinine.) The readings of the colorimeter, of which several should be taken, should be completed within twenty minutes of the dilution, as the reaction liquid frequently fades. The zero of both sides of the colorimeter should be tested, and it is as well to test the use of the colorimeter by employing the standard solution on both sides before determining the creatinine. The readings in the creatinine determination should not differ by more than 0.3 mm. If the average reading is less than 5 mm., the urine should be carefully diluted and another determination made; if above 13 mm., 20 c.c. urine instead of 10 c.c. should be employed.

The result of the determination is calculated from the formula :—

$$x = \frac{10 \times 8.1}{a}$$

Where  $x$  is the quantity of creatinine in milligrammes in the volume of urine employed, and  $a$  is the colorimeter reading in millimetres. The amount of creatinine is inversely proportional to the colorimeter reading. The formula depends on the fact that, when 10 mg. of pure creatinine was employed for a determination, the colorimeter reading, against 8 mm. of standard bichromate, was 8.1 mm.

To get rid of aceto-acetic acid, if present. Graham and Poulton have shown that aceto-acetic acid is converted by heat into acetone which

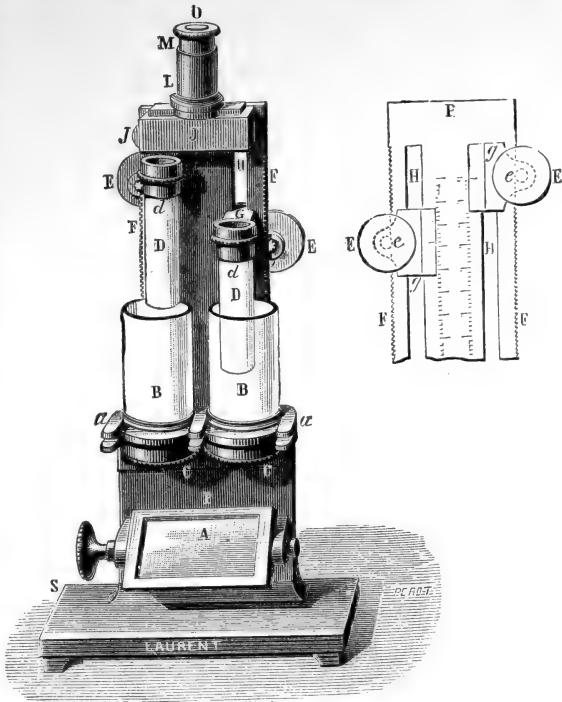


FIG. 197.—The Dubosq Colorimeter.

can then be readily removed. To 10 c.c. of the urine to be tested add 1 c.c. of a 10 per cent. solution of phosphoric acid. The test tube is then fitted with a cork carrying a capillary tube and outlet tube, put into a water bath at 65°–70°, connect with the water-pump and run water so as to maintain a pressure of about 210 mm. of mercury. Allow to run for about three-quarters of an hour. Temperature of bath not to exceed 70°. At the end of the heating period, stop suction, cool the test tube under the tap, add a sufficient amount of previously standardised soda solution to neutralise exactly the phosphoric acid added, then transfer to a measuring cylinder and make up to 20 c.c.; 2 c.c. of the solution correspond to 1 c.c. of the urine.

**Estimation of Creatine.**—Place in a small flask 10 c.c. urine and 5 c.c. N. HCl.

Cover the mouth of the flask with tinfoil and heat in the autoclave at 115°–120° for twenty minutes. If no autoclave is available the flask with small filter funnel condenser may be heated on a water bath for four to five hours. Cool to room temperature. Add sufficient caustic soda to neutralise the acid added, 15 c.c. picric acid solution and 5 c.c. 10 per cent. caustic soda. Allow to stand for five minutes. Wash the contents of the flask into a 500 c.c. flask, make up to

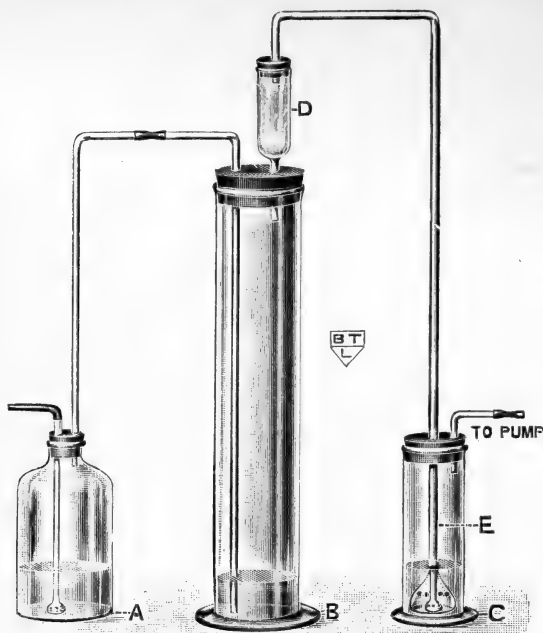


FIG. 198.—Folin's apparatus for estimating ammonia.

500 c.c., and proceed as for creatinine. The difference between this result and that for creatinine previously determined represents the amount of creatine present.

**Estimation of Ammonia.** *Folin Method.*—25 c.c. urine are placed in a long tube or cylinder, add 8–10 gms. sodium chloride or potassium oxalate and 5 c.c. liquid paraffin (to prevent excessive frothing) and finally 1 gm. anhydrous sodium carbonate. The tube or cylinder is connected on one side with the air, which may be first passed through a wash bottle, and on the other side with a flask or tube containing 20 c.c.

$\frac{N}{10}$   $H_2SO_4$  to absorb the ammonia liberated. It is well to add a few

drops of a 1 per cent. solution of alizarin red to the  $\frac{N}{10}$  acid to act as indicator in case more ammonia is liberated than the amount of acid used can absorb. Air is then made to pass rapidly through the urine either by means of a blower or a good suction pump for one and a half to two hours. The absorbing acid can then be titrated as usual with  $\frac{N}{10}$  NaOH. 1 c.c.  $\frac{N}{10}$   $H_2SO_4 = 0.0017$  gm.  $NH_3$ .

The vacuum distillation methods of estimating ammonia in urine are more accurate than Folin's and much more rapid.

Many methods have been used. The method here described is the one introduced by Shaffer. Place 50 c.c. urine in a round-bottom  $\frac{1}{2}$  litre flask A (Fig. 199), add 20 gms. sodium chloride to prevent

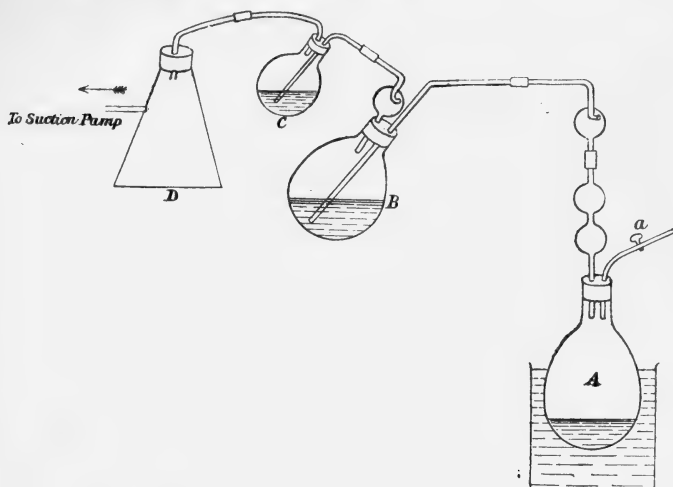


FIG. 199.—Shaffer's method of estimating ammonia in urine.

decomposition and 50 c.c. methyl alcohol to reduce the boiling point of the mixture. In flask B place 50 c.c. or less  $\frac{N}{10}$  acid and in C 10 c.c.

$\frac{N}{10}$  acid, diluted in both cases with a little water. The flasks may be tilted obliquely, and should be large enough to prevent loss of acid by spraying during the violent commotion which is set up by the rapid passage of steam. If such loss should occur, the acid may be recovered by rinsing out the flask D. When the apparatus is ready, 1 gm. of dry sodium carbonate is added to the liquid in the flask A, the stopper is rapidly inserted and the suction started. The pump will quickly reduce the pressure to about 30 mm., and the liquid in A, which is warmed up to about  $40^\circ C.$  in a water bath, will begin to boil. The temperature of the bath must be maintained and should not be allowed to rise above  $50^\circ C.$  for fear of decomposing urea. When the boiling has continued for fifteen minutes, all the ammonia will have been given off and the operation is stopped by slowly letting in air by the stop-cock *a*.

The acid in B and C is titrated, after a few drops of a 1 per cent. solution of alizarin red have been added as the indicator. Instead of adding the alcohol all at once a side tube with stop-cock may be connected with flask A so that alcohol can be added as required to keep excessive frothing in check.

**Estimation of Amino Acids.** *Sørensen's Method.*—Measure 50 c.c. urine into a 100 c.c. measuring flask, then add 1 c.c. phenolphthalein solution (0.5 per cent.) and 2 gms. solid  $\text{BaCl}_2$ . After shaking, add a saturated solution of baryta until a definite red colour is produced and then 5 c.c. in addition. Fill up to the mark with water, shake well and allow to stand at least fifteen minutes before filtering through a dry filter.

80 c.c. (= 40 c.c. urine) of the clear red filtrate are placed in another 100 c.c. measuring flask and  $\frac{N}{5}$  HCl added until the solution is definitely acid to litmus then filled up to the mark with water. Of this solution two lots, A and B, of 40 c.c. are taken, A for estimation of the ammonia either by Folin's method or preferably by the vacuum distillation method, and B for the estimation of ammonia plus amino acids. This estimation is carried out as follows:—Add  $\frac{N}{5}$  NaOH until the fluid is neutral, using phenolphthalein as indicator; then add 20 c.c. neutral commercial formalin and titrate again with  $\frac{N}{5}$  NaOH until a faint but definite rose colour is developed. Then add 2 drops more of the alkali. End point—deep red.

Calculation.—c.c.  $\frac{N}{5}$  NaOH required after the addition of the formalin  $\times 2.8 =$  mgm. ammonia + amino acid nitrogen in 16 c.c. urine. Multiply by 6.25 to convert to per cent.

Then B result - A result = amino acid nitrogen.

**Estimation of Chlorides.** *Volhard's Method.*—Pipette 10 c.c. albumin-free urine into a 100 c.c. graduated flask, add 5 c.c. nitric acid and 20–30 c.c.  $\frac{N}{10}$  silver nitrate solution (17 gms. per litre) measured accurately with a pipette. The silver solution must be in excess. Add distilled water to the 100 c.c. mark and mix thoroughly. Allow to stand for a few minutes, then filter through a dry chloride-free filter paper into a dry clean beaker and of the filtrate take 50 c.c. into a flask, add 10–20 c.c. 10 per cent. iron alum solution and titrate with  $\frac{N}{10}$  potassium sulphocyanide (9.73 gms. per litre) until a permanent red colour results.

$$1 \text{ c.c. } \frac{N}{10} \text{ AgNO}_3 \text{ solution} = 0.00585 \text{ gm. NaCl.}$$

In this method it is the amount of silver nitrate left uncombined which is determined, and this amount subtracted from the amount of silver nitrate originally added gives the amount of chloride present.

**Inorganic Phosphates.**—10 c.c. urine are diluted to 40 c.c. with water, an excess (10 c.c.) of magnesium citrate mixture added and the whole made distinctly alkaline with ammonia. Stir well and allow to stand overnight. Filter off the precipitate of ammonium magnesium phos-



phate which has formed through a small filter, wash well with dilute ammonia (about 1 per cent.) and dry at 100°. To estimate the  $P_2O_5$  present as pyrophosphate the precipitate and filter paper are incinerated in a weighed crucible heating, after initial burning is complete, to bright redness for fifteen to thirty minutes. This converts the ammonium magnesium phosphate into magnesium pyrophosphate. Crucible is cooled and weighed.

$$\text{Magnesium pyrophosphate} \times 0.638 = P_2O_5.$$

**Magnesium Citrate Mixture.**—Dissolve 400 gms. citric acid in 500 c.c. water by the aid of heat. Add to the hot solution 20 gm. magnesium oxide (light). Cool, add 400 c.c. of 0.880 ammonia and then water to 1,500 c.c. Allow to stand twelve to twenty-four hours and filter.

**Total Phosphates.** *Neumann's Wet Ash Method.*—10 c.c. urine are placed in a 500 c.c. combustion flask and 10 c.c. concentrated sulphuric acid plus 5 c.c. concentrated nitric acid are added. The mixture is then carefully heated in a fume chamber until contents are colourless. If there is much delay in the decolorisation, 3–4 c.c. of nitric acid may be cautiously added and the flask again heated. When oxidation is complete allow to cool, add 150 c.c. water and 30 c.c. ammonium nitrate solution (50 per cent.), put on a water bath and heat to about 75° (as hot as the hand will bear), and then add 10–20 c.c. ammonium molybdate solution (10 per cent.). Shake for about two minutes and return to the water bath until the yellow precipitate of ammonium phosphomolybdate settles (takes about half an hour). Filter off the yellow precipitate with aid of suction through asbestos in a Gooch crucible, wash rapidly and well with 1 per cent. nitric acid, dry in a hot-water oven, cool and weigh.

0.1 gm. Am. phosphomolybdate = 0.00374 gm.  $P_2O_5$  or 0.00163 gm. P.

If preferred the precipitate of phosphomolybdate, washed free from acid, is dissolved in a known volume of  $\frac{N}{2}$  NaOH, solution washed into the original combustion flask with about 200 c.c. of water, a few drops of phenolphthalein solution added and the solution boiled for about fifteen minutes to get rid of ammonia (test the steam with glazed litmus paper). Cool rapidly in running water and titrate excess of alkali with  $\frac{N}{2}$   $H_2SO_4$ .

$$1 \text{ c.c. } \frac{N}{2} H_2SO_4 = 1.268 \text{ mg. } P_2O_5 \text{ or } 0.5536 \text{ mg. P.}$$

Total phosphates less inorganic phosphates gives the amount of organic phosphate present.

**NOTE.**—Protein must first be removed if present. This is best done by boiling the urine acidified with acetic acid in a flask, cooling and filtering.

**Inorganic Sulphates** (Folin).—25 c.c. of urine are diluted with 100 c.c. of water in an Erlenmeyer flask (of 250 c.c. capacity) and 10 c.c. of dilute hydrochloric acid (1 part HCl (conc.) to 4 parts water) added. A burette containing a 5 per cent. solution of barium chloride is then placed over the mouth of the flask and 10 c.c. of the reagent allowed to drop into the contents of the flask at a slow

rate (not quicker than 5 c.c. per minute).<sup>1</sup> The flask must not be moved until after the end of an hour, when it is shaken and the precipitate collected on an asbestos mat in a Gooch crucible, washed with about 250 c.c. cold water, then with alcohol and ether, dried and ignited. In doing this, the flame must not be applied directly to the perforated bottom of the crucible, but the Gooch crucible must be placed in a large nickel crucible. The crucible must also be covered with a lid during the ignition. Ten minutes' ignition is usually sufficient; precipitate should be quite white. Cool and weigh.

**Total Sulphates (Inorganic and Ethereal) (Folin).**—25 c.c. urine are mixed with 20 c.c. of dilute hydrochloric acid (1:4) in an Erlenmeyer flask of about 250 c.c. capacity, and, after covering the mouth of the flask with a watch-glass in a small filter funnel, gently boiled for twenty to thirty minutes. The flask is then cooled in running water, its contents diluted with distilled water to about 150 c.c. and 10 c.c. of 5 per cent. solution of barium chloride added, and the further procedure followed as above described.



Total sulphates—inorganic sulphates = ethereal sulphates.

**Total Sulphur. Modified Benedict Method (Denis).**—Place 10 c.c. of urine in a small (about 7 cm. diam.) porcelain evaporating basin and 5 c.c. of copper nitrate reagent (125 gms. copper nitrate (sulphate free); 125 gms. sodium chloride; 50 gms. ammonium nitrate: distilled water to 500 c.c.). Evaporate on the waterbath (or over a very small flame) to dryness. When quite dry heat the residue gently with a small flame until it is blackened. The flame is then gradually increased and the basin is heated to redness for ten minutes after the black residue, which first fuses, has become dry. Allow the basin to cool, add 10–20 c.c. dilute HCl, and warm gently till complete solution of the contents takes place. Transfer to a beaker, dilute with cold water to 100–150 c.c., add 10 c.c. 10 per cent. BaCl<sub>2</sub> solution and continue as in the sulphate estimations.

Total sulphur less total sulphates = neutral sulphur.

If special accuracy is required an alcohol flame must be used for heating instead of gas as gas contains much sulphur and may thus give too high results.

*Volumetric Method of Estimating Sulphates.*—(Rosenheim and Drummond's benzidine method.)

This method depends upon the insolubility of benzidine sulphate in hydrochloric acid solution. Ethereal sulphates may be determined by this method after hydrolysis of the ethereal sulphates by hydrochloric acid as in the Folin determination. Total sulphates less inorganic sulphates = ethereal sulphates.

20 c.c. of urine are placed in a 250 c.c. flask and acidified with dilute HCl (1:4) until the reaction is distinctly acid to Congo red, then 100 c.c. of benzidine<sup>2</sup> solution is run in. A precipitate forms in a few seconds, and after standing ten to fifteen minutes it is filtered under pressure, care being taken not to allow the precipitate to be

<sup>1</sup> More rapid addition of the reagent causes the results to be too high, i.e. produces an impure precipitate.

<sup>2</sup> Benzidine solution. Rub 4 gms. of benzidine into a paste with water, transfer it with about 500 c.c. water into a 2 litre flask. 5 c.c. conc. HCl are added and the volume made up to 2,000 c.c. with water.

sucked dry at any time on the filter. Precipitate is washed with 10–20 c.c. water saturated with benzidine sulphate. The precipitate and filter paper are then transferred into the original precipitation flask with about 50 c.c. water and titrated with  $\frac{N}{10}$  NaOH using phenolphthalein as indicator.

$$1 \text{ c.c. } \frac{N}{10} \text{ NaOH} = 0.0049 \text{ gm. H}_2\text{SO}_4.$$

This method may also be employed to estimate the sulphate present in the neutral sulphur determination. The solution which results on treatment of the black residue with dilute HCl may be treated with benzidine solution as above and the sulphate determined volumetrically.

**Estimation of Phenols in Urine** (Folin and Denis slightly modified).—Place 10 c.c. of ordinary or 20 c.c. of very dilute urine in a 50 c.c. volumetric flask. Add 2–10 c.c. acid silver lactate solution (5 per cent. silver lactate in 5 per cent. lactic acid solution) until no more precipitate is obtained, then add a few drops of colloidal iron and shake. Fill to the mark with distilled water, shake again and filter. This precipitation removes uric acid and traces of protein quantitatively. Transfer 25 c.c. of the filtrate to a 50 c.c. volumetric flask and to it add a sufficient quantity of saturated sodium chloride solution (containing 10 c.c. conc. HCl per litre) to precipitate all the silver. Fill to the mark with distilled water and filter.

To determine “free” (non-conjugated) phenols take 20 c.c. of the filtrate, place in a 50 c.c. flask, add 5 c.c. of the phosphotungstic phosphomolybdic acid reagent<sup>1</sup> and 15 c.c. saturated sodium carbonate solution. Dilute to mark with water at 30–35 C. and allow to stand for twenty minutes. Estimate deep blue solution in a Dubosecq colorimeter against a standard solution of phenol.

Total (free and conjugated) phenols are determined by transferring 20 c.c. of the same filtrate to a large test tube, add 10 drops of conc. HCl, place small funnel in mouth of tube and then heat rapidly to boiling over a free flame. Now place in a boiling water bath for ten minutes. At the end of this time remove, cool, transfer contents to a 100 c.c. measuring flask, add 10 c.c. of the reagent<sup>1</sup> and 25 c.c. of the saturated sodium carbonate solution. Make up to volume, shake and let stand for twenty minutes. Read against a standard solution of phenol.

Standard solution of phenol is a solution of pure phenol 10 mg. in 100 c.c.  $\frac{N}{100}$  HCl. Dissolve 0.100 gm. crystallised phenol in 100 c.c.

$\frac{N}{10}$  HCl. Take 25 c.c. of this solution in a 250 c.c. flask, add 50 c.c.  $\frac{N}{10}$  NaOH, heat to 65° C., add 25 c.c.  $\frac{N}{10}$  iodine solution, stopper,

<sup>1</sup> Reagent. Transfer to a large flask 34 gms. of ammonium molybdate  $[(\text{NH}_4)_6(\text{MoO}_4)]$  (or 25 gms.  $\text{MoO}_3$ ), add 140 c.c. of 10 per cent. NaOH and about 150 c.c. of water. Boil for twenty minutes to get rid of ammonia, then add to the solution 100 gms. sodium tungstate, 50 c.c. of 85 per cent. phosphoric acid and 100 c.c. of conc. HCl. Dilute to a volume of 700–800 c.c., close the mouth of the flask with a funnel and watch glass and then boil gently for not less than four hours, adding hot water from time to time to replace that lost during boiling. Cool and dilute to 1,000 c.c.

let stand at room temperature for thirty to forty minutes. Add 5 c.c. conc. HCl and titrate excess of iodine with  $\frac{N}{10}$  sodium thiosulphate solution. 1 c.c. of  $\frac{N}{10}$  iodine solution = 1.567 mg. phenol. On the basis of the results dilute phenol solution so that 10 c.c. = 1 mg. of phenol. (Benedict and Theis (*J. Biol. Chem.* 36, 1918) recommend resorcinol as standard as the solution is easily prepared and keeps well.) Add to 10 c.c. of this standard in a 100 c.c. flask 0.5 c.c. HCl and 10 c.c. of the silver lactate-lactic acid solution (as lactic acid also gives a blue colour with the reagent), shake well and filter. To the filtrate add 10 c.c. phenol reagent<sup>1</sup> and 25 c.c. saturated sodium carbonate solution. (For free phenols best 5 c.c. reagent and 15 c.c. carbonate solution.) Fill to the 100 c.c. mark with water about 30° C. and allow to stand about twenty minutes. Set standard in Duboseq at 20 mm.

**Estimation of Sugar.** *Pavy's Method.*—The standard solution contains 120 c.c. Fehling's solution and 300 c.c. strong ammonia per litre.

The nozzle of a burette is fitted to a small round-bottomed flask by means of a cork through which is also passed a short bent tube to allow of the escape of steam and ammonia, when the flask is boiled. The urine is diluted exactly 10 to 50 times according to the amount of sugar present. The burette is filled with diluted urine, care being taken to see that there are no bubbles in the nozzle. 10 c.c. of Pavy's solution and about an equal volume of water are placed in the flask. The flask is now heated till it boils. The heating is continued and the urine allowed to drop in from the burette at such a rate that boiling does not cease. When the colour of the solution in the flask is perceptibly less the rate of addition of drops is reduced, but is continued until all the blue colour has disappeared. The first reading will be almost certainly too high so that repeat determinations must be made. In the later determinations it is recommended to run in fairly rapidly the quantity of urine which will almost suffice, then wait till the colour is constant before adding the rest of the urine required *drop by drop* till the blue colour has completely disappeared. The amount of diluted urine employed should not be less than 2 c.c. or more than 5 c.c. Calculation: 10 c.c. Pavy solution are equivalent to 0.005 gm. dextrose.

**Quantitative Estimation of Beta-Oxybutyric Acid, Acetoacetic Acid and Acetone** (van Slyke).—In this method all three substances—total acetone bodies—can be determined at once, a separate determination of the beta oxybutyric acid can be made by boiling off the acetoacetic acid and acetone from the treated urine which has been acidified with sulphuric acid.

Place 25 c.c. of the urine to be tested in a 250 c.c. measuring flask, add 100 c.c. distilled water, 50 c.c. copper sulphate solution (200 gms. copper sulphate,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , dissolved in water and made up to 1,000 c.c.) and mix well. Next add 50 c.c. of a well-shaken suspension of calcium hydroxide (100 gms. light calcium hydroxide in 1,000 c.c. water) and shake the whole mixture well. The copper and lime are added to remove sugar and other interfering substances. If the urine contains more than 8 per cent. sugar it must be diluted so that the sugar present does not exceed this amount. The reaction of the

<sup>1</sup> See footnote on page 291.

mixture should be definitely alkaline to litmus, if not add more calcium hydroxide. Dilute to the 250 c.c. mark and allow to stand for at least thirty minutes and then filter through a dry folded filter paper into a dry flask. Some of the filtrate may be tested now to see if all sugar is removed by boiling. If sugar is present a yellow precipitate will be obtained instead of a white precipitate.

25 c.c. of the filtrate is now measured into a 500 c.c. Erlenmeyer flask, 100 c.c. water, 10 c.c. 17N. sulphuric acid (add 500 c.c. conc.  $\text{H}_2\text{SO}_4$  cautiously to 450 c.c. distilled water, cool thoroughly and make up to 1,000 c.c. with water; the solution may require slight adjustment) and 35 c.c. mercuric sulphate solution (73 gms. pure red mercuric oxide dissolved in 1,000 c.c. of 4N. sulphuric acid). Connect a good reflux condenser, best with straight condensing tube, to the Erlenmeyer and heat to boiling. When boiling has properly started add 5 c.c. bichromate solution (50 gms. potassium bichromate dissolved in water and made up to 1,000 c.c.) through the condenser tube; continue gentle boiling for one and a half hours. Then cool and filter off the yellow precipitate through asbestos in a weighed Gooch crucible. Wash out the Erlenmeyer with about 200 c.c. cold water. Suck asbestos dry and then transfer to a hot air oven at  $110^\circ$  for an hour. Cool in room air and weigh.

Calculation. Assuming that beta-oxybutyric acid forms 75 per cent. of the total acetone bodies present (the usual proportion), then if 25 c.c. of the filtrate, i.e. 2.5 c.c. of original urine, are used, 1 gm. of precipitate equals 2.48 gms. total acetone bodies as acetone per 100 c.c. urine.

Instead of weighing the precipitate the contents of the Gooch, including the asbestos, may be washed into a small beaker with as little water as possible. Add 15 c.c.  $\frac{N}{1}$  HCl and heat the mixture until all precipitate is dissolved. Cool the solution, add 6-7 c.c. of 3M. sodium acetate solution and then run in  $\frac{M}{5}$ .KI rapidly from a burette with constant stirring. If more than a small amount of mercury is present a red precipitate of  $\text{HgI}_2$  at once forms and redissolves as soon as 2-3 c.c. of KI in excess of the amount required to form the soluble  $\text{K}_2\text{HgI}_4$  have been added. If only a few mg. of Hg are present the excess  $\text{K}_2\text{I}$  may be added before the  $\text{HgI}_2$  has had time to precipitate, so that the titrated solution remains clear. In this case not less than 5 c.c. of the  $\frac{M}{5}$ .KI are added as final titration is not satisfactory if less is present. The excess KI is titrated back by adding  $\frac{M}{20}$ . $\text{HgCl}_2$  from another burette until a permanent red precipitate forms. As the reaction is  $\text{HgCl}_2 + 4\text{KI} = \text{K}_2\text{HgI}_4 + 2\text{KCl}$ . 1 c.c. of  $\frac{M}{20}$ . $\text{HgCl}_2 = 1$  c.c.  $\frac{M}{5}$ .KI; 1 c.c. of  $\frac{M}{5}$ .KI = 13 mg. mercury acetone precipitate.

When 25 c.c. filtrate, i.e. 2.5 c.c. urine, are used 1 c.c.  $\frac{M}{5}$ .KI = 0.032g total acetone bodies, or 0.034g beta-oxybutyric acid as acetone per 100 c.c. urine. Standardise the  $\frac{M}{20}$ . $\text{HgCl}_2$  by the sulphide precipitation method.

As mentioned above, beta-oxybutyric acid may be estimated separately by previous removal of the aceto-acetic acid and acetone. Take 25 c.c. of the filtrate from the copper lime precipitation in an open flask, add 100 c.c. water and 2 c.c. 17N. sulphuric. Boil for ten minutes over the free flame. Cool, measure, return to flask, make up volume to 127 c.c., add 8 c.c. of the sulphuric acid and 35 c.c. of the mercuric sulphate. Connect with reflux condenser and then proceed as before.

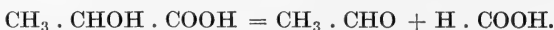
1 gm. precipitate = 2.64 gms. beta-oxybutyric per 100 c.c. as acetone. Beta-oxybutyric acid = acetone value  $\times$  1.793.

Van Slyke states that there should be a preliminary test of the materials, using distilled water in place of urine. No precipitate of any kind should be formed.

A good rough approximation to the amount of aceto-acetic acid and acetone present may be obtained by using a modified ammonia estimation apparatus. Put 5-10 c.c. urine in a large boiling test tube fitted with appropriate rubber stopper, add 2-3 drops 5 per cent. sulphuric acid, connect with an absorption tube or flask containing 5-10 c.c. Scott-Wilson cyanide reagent.<sup>1</sup> Place tube containing the urine in a waterbath at 70°-75° and start suction pump so as to run a moderately rapid air stream through the urine. If acetone is present, even if only in traces, the reagent becomes turbid. The degree of turbidity may be compared with that given by a standard solution of acetone containing 0.5 mg. in a nephelometer or Duboscq colorimeter. (See Marriott, *J. Biol. Chem.*, 16 and 18; Folin and Denis, *J. Biol. Chem.*, 18.)

**Quantitative Estimation of Lactic Acid (Ryffel).**—Lactic acid can be shown to be excreted in minute amount, 0.004 gm. per hour during the day and about half this at night by a perfectly normal man on an ordinary diet. If the man is made to perform strenuous work there may be a very considerable rise in the output, so much indeed that it may be determined roughly by the thiophene test. Render the urine alkaline with sodium carbonate, evaporate, extract with alcohol, evaporate alcohol, dissolve residue in a little water, strongly acidify with phosphoric acid and then thoroughly extract with ether. Ether is shaken up with dilute sodium carbonate solution, the alkaline solution decolorised by boiling with charcoal, filtered and evaporated to dryness. Residue dissolved in 5 c.c. conc. H<sub>2</sub>SO<sub>4</sub> and the thiophene test applied (see p. 230).

The distillation method devised by Ryffel gives good results even when only small amounts are to be dealt with. This method depends on the fact that lactic acid, when heated above 140° C. with sulphuric acid, yields acetaldehyde quantitatively according to the following equation:—



40 c.c. of the liquid, which must be free from sugar and nearly free from protein, are placed in a 500 c.c. Jena distillation flask. 45 c.c.

<sup>1</sup> Reagent. Dissolve 10 gms. mercuric cyanide in 600 c.c. water and add to this solution 180 gms. pure caustic soda also dissolved in 600 c.c. water. Pour in a slow stream, stirring vigorously, into the mixture 400 c.c. of water containing 2.9 gms. silver nitrate. If properly made the silver dissolves completely, giving a clear solution. Usually there is slight turbidity; set aside for three to four days to settle; decant off clear supernatant liquid. When reagent stands a new sediment gradually forms so that solution deteriorates slowly. Will keep good several months.

pure sulphuric acid are rapidly added from a dropping funnel, the flask being shaken and cooled under the tap. The flask is then fitted with a rubber cork carrying an inlet tube for steam and a thermometer, so arranged that both dip well below the surface of the liquid. It is then placed in a slanting position on wire gauze on a retort stand and attached to a good vertical condenser. (For this purpose the exit tube of the flask must be bent at a suitable angle.) A flask of about 300 c.c. capacity, immersed in cold water, is placed as the receiver of the condenser with its mouth just touching the jacket of the condenser, so as to prevent loss of aldehyde by evaporation. A gentle current of steam from an ordinary steam generator is then passed into the distillation flask, which is vigorously heated with a Bunsen burner. Distillation will generally begin at about 140° C., but the heating is continued till the temperature reaches 155° C., when the current of steam is increased, and the heat applied to the flask adjusted so that the temperature is kept between 153° and 157° C. When about 100 c.c. have collected in the receiver, or the distillation has lasted nearly thirty minutes, the decomposition is complete. The contents of the receiver are rendered just permanently alkaline by the addition of 2 per cent. caustic soda solution and a little litmus solution, diluted to about 150 c.c., and redistilled into a flask with a 100 c.c. mark in the neck, using the same precautions to prevent loss as before, until about 50 c.c. have been collected. (When the amount of lactic acid is excessively small, as is the case in normal urine, a 50 c.c. flask may be employed, the quantities given in what follows being halved.)

To the second distillate are added 0.5 c.c. Schiff's reagent (see later) and water to bring the volume to 100 c.c. The flask is stoppered, inverted a few times to mix its contents, placed in a glass vessel containing water at 15° C., and left for thirty minutes in diffuse daylight. The Schiff's reagent reacts with the aldehyde present, giving a red colour, which reaches a maximum in thirty minutes and then slowly fades. This reaction may be used qualitatively as a test for lactic acid. For quantitative estimation the coloured liquid (*a*) is transferred at the end of thirty minutes to one tube of a colorimeter. A convenient depth of liquid is selected. The two formaldehyde standards (see later) are selected which are nearest to *a* in colour, and the depth of each determined which gives the same intensity of colour as the selected depth of *a*.

The calculation is best described by an example.

	Formaldehyde 4 c.c.	<i>a</i>	Formaldehyde 3 c.c.
Readings of equal depth of colour,	2.42 cm.	2 cm.	1.46 cm.
10 ÷ readings,	4.13	5	6.85

$$\text{Then } a \text{ is equivalent to } 3 \text{ c.c. } + \frac{5 - 4.13}{6.85 - 4.13}$$

$$= 3.32 \text{ c.c. standard formaldehyde solution.}$$

The amount of lactic acid in the liquid originally employed =

$$\frac{3.32 \times 3.435 \times n}{0.4} \text{ mg.}$$

where *n* is the standard value of the formaldehyde.

If the colour of *a* is much greater than that of any of the standards, another determination must be made, using a more dilute solution of lactic acid.

*The Formaldehyde Standards.*—A series of four stoppered flasks is

prepared containing 0.5 c.c. Schiff's reagent and 1.5 c.c., 2 c.c., 3 c.c., 5 c.c. respectively of dilute standard formaldehyde solution, made up to 100 c.c. with water. These are placed in a dark cupboard till required. The colour develops very slowly, and is fairly permanent, so that the standards may be used any time within three days after the first twelve hours.

*The Dilute Standards Formaldehyde Solution.*—10 c.c. commercial formalin (40 per cent. formaldehyde) are diluted to 100 c.c. This solution will keep practically indefinitely. To make the dilute standard solution 5 c.c. of this solution are diluted to 500 c.c. This dilute solution will keep practically unaltered for a week if well stoppered. It is standardised, unless made from an already standardised formaldehyde solution, by the following method: 40 c.c. are measured into a stoppered bottle, 25 c.c.  $\frac{N}{10}$  iodine solution are added, and then 10 per cent. caustic soda, till the liquid assumes a light yellow colour. The mixture after standing for ten minutes is acidified with dilute hydrochloric acid and titrated with  $\frac{N}{10}$  sodium thiosulphate solution, until the colour of the iodine just disappears. The volume in c.c. of thiosulphate solution required is subtracted from 25 c.c. Let the remainder =  $b$  c.c. Then the formaldehyde in mg. present in 1 c.c. of the solution =  $n = \frac{1.49 \times b}{40}$ .

The value of  $n$  should be nearly 0.4 mg.

*Schiff's Reagent.*—1 gm. finely powdered rosaniline hydrochloride and 100 c.c. water are placed in a small bottle with a closely fitting stopper. Sulphur dioxide is passed in from a syphon, till the dye just dissolves to a yellow solution, when the liquid is very nearly saturated with the gas. The reagent loses sulphur dioxide rather readily, so that it must be kept closely stoppered, and must be resaturated occasionally with sulphur dioxide. The formaldehyde standard with 0.5 c.c. of the reagent and 5 c.c. dilute formaldehyde solution (2 mg. formaldehyde) made up to 100 c.c. with water should be of such a depth of colour, that by the colorimeter 1.3–1.7 cm. is equivalent in colour to 0.7 cm.  $\frac{N}{100}$  potassium permanganate.

The method can be applied to urine either directly, or after rendering alkaline with sodium carbonate and evaporating on the waterbath, but not more than 40 c.c. of urine of specific gravity 1,020 should be employed for one distillation in either case, as with more urine frothing is liable to occur.

Glycuronic acid forms a source of error, but may be removed by means of basic lead acetate. For this purpose 25–200 c.c. urine are measured into a 500 c.c. graduated flask. Slight excess of basic lead acetate solution, 10 c.c. strong ammonia and water to make 500 c.c. are added. The contents of the flask are well mixed, allowed to stand for a short time and filtered through a dry filter into a dry flask. A measured volume of the filtrate (350 c.c. or less) is evaporated in a dish on the waterbath, sodium carbonate solution being added to keep the liquid alkaline. The residue in the dish is then washed into the distillation flask with 40 c.c. water and 45 c.c. sulphuric acid and treated as above. This treatment causes a small loss of lactic acid, so that only about 50 per cent. of minute quantities of lactic acid added to urine



are recovered. When the quantity of lactic acid is considerable, however, the loss is negligible.

In order to apply the method to blood the following preliminary procedure is necessary. The blood, of which 20 c.c. is usually quite sufficient, is diluted about five times, heated to boiling in order to coagulate the proteins, and filtered. The coagulum is very thoroughly washed with boiling, faintly acidulated water. The total liquid thus obtained is rendered alkaline with sodium carbonate, evaporated and employed for the determination.

**Estimation of Urochrome.**—Add about 20 gms. finely powdered ammonium sulphate to 25 c.c. urine. Actively stir the mixture with a glass rod so that most of the ammonium sulphate is dissolved. Allow to stand for at least thirty minutes in order that other pigments present may precipitate, then fill up to 50 c.c. with hot saturated ammonium sulphate solution and filter at once. 10 c.c. of the filtrate is placed in one of the cups of a Duboscq colorimeter and compared with a standard solution of "Echtgelb" (Müller, Leipzig) containing 0.01g. Echtgelb in 2 litres of water.

**Urobilin.**—Urobilin is a derivative of hæmoglobin and is probably identical with the faecal pigment stercobilin. It readily combines with alkalis to form salts which are easily soluble in water. Urobilin shows a well-marked absorption band in the green near F. If urine is first rendered acid with a mineral acid or if a drop or two of tincture of iodine be added the chances of getting the spectrum in urine are much increased. Its presence can also be detected in urine by its fluorescence, particularly in the presence of zinc salts.

*The Schlesinger Test.* Take 10–15 c.c. urine in a wide test tube and add to it an equal volume of *well-shaken* zinc acetate solution,<sup>1</sup> mix well and allow to stand for twelve to twenty-four hours in order to allow the precipitate to settle. The clear supernatant fluid of urobilin, if present, will show a beautiful green fluorescence. A better result is obtained by filtering off the precipitate and examining the column of fluid for fluorescence from above. If the column appears colourless no urobilin is present, or only a minute amount, but if it appear yellowish red or rose it may be regarded as pathological.

A more refined method of carrying out this test is to add 3–4 drops glacial acetic acid to 50 c.c. urine, rendering the reaction definitely acid. Then drop by drop, shaking well, 1 per cent. tincture of iodine (1 drop to each 2 c.c. of urine). Add 5 c.c. thymol chloroform and shake thoroughly. The chloroform extract is mixed with an equal volume of an alcoholic zinc acetate solution (alcohol 93 per cent. 500 c.c., zinc acetate 3 gms., glacial acetic acid 2 c.c.; filter before use), shake and filter. The presence of a beautiful green fluorescence is indicative of the presence of urobilin.

**Preparation of Urobilin** (Garrod and Hopkins).—Saturate the urine with ammonium chloride in order to remove uric acid, filter, acidify the filtrate with sulphuric acid, saturate with ammonium sulphate. Extract this mixture in a large separating funnel with an equal volume of a mixture of 1 part chloroform and 2 parts ether. The chloroform ether extracts the urobilin. The extract is then shaken with faintly alkaline water which takes up the urobilin. In order to purify the

<sup>1</sup> 10 gms. of zinc acetate are added to 100 c.c. alcohol. Most of the acetate remains undissolved, hence the reagent must be well shaken before use.

urobilin the watery solution is again saturated with ammonium sulphate, after all the ether has been removed by passing a rapid current of air through the solution, and the process of extraction with chloroform ether repeated. To get the urobilin from the chloroform ether shake with a small amount of water containing a little ammonia, acidify the alkaline extract to precipitate the urobilin, take this urobilin up in a small quantity of pure chloroform and on the evaporation of the chloroform urobilin is left. If it contains any ammonium sulphate this may be got rid of by dissolving the urobilin in absolute alcohol, filtering and finally evaporating the alcohol. If hydrochloric acid be added to a solution of this urobilin in caustic soda until the reaction is just acid a turbid fluid results. Spectroscopic examination shows in

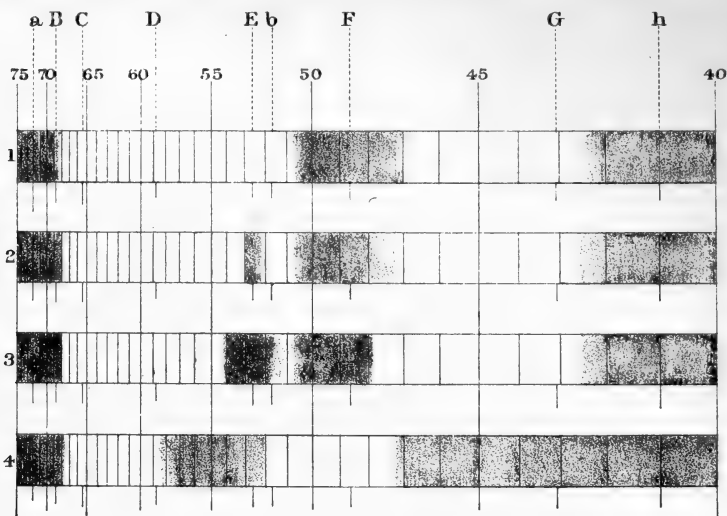


FIG. 200.—Pigments of urine.

1. Acid urobilin in strong solution.
2. Urobilin precipitated by acid from its alkaline solution and partially redissolved. The so-called E-band spectrum.
3. Uroerythrin.
4. Uroerythrin in pink urate sediments.

addition to the characteristic band at F, another band near the E line. This band disappears if the liquid be filtered.

Another pigment which is present in urine is **uroerythrin**. This pigment is the colouring matter present in pink urate sediments. The pigment may be separated from the urates. Dissolve the pink urates in warm water, saturate with ammonium chloride. Filter, extract precipitate with alcohol, shake the alcoholic extract with chloroform after the addition of a drop of acetic acid. The chloroform extract will show on spectroscopic examination the characteristic bands (rather ill-defined) near E and F.

**Hæmatoporphyrin** is another pigment which probably constantly occurs in normal urine in very small amount. The output may rise after the administration of drugs like sulphonal, trional, etc., and sometimes in lead poisoning. It may exist, in part at least, in the urine

in chromogen form. Hæmatoporphyrin possesses a well-marked spectrum which differs according to whether the preparation is acid or alkaline (see pp. 247 and 250). It is impossible to detect hæmatoporphyrin spectroscopically in the urine unless it be present in fairly large amount; when present, even in acid urine, it is the alkaline spectrum which is obtained. It may be obtained from the urine by adding to each 100 c.c. urine 20 c.c. 10 per cent. caustic soda. The precipitate is filtered off, washed, and then treated with acid alcohol. The solution in acid alcohol gives the acid spectrum, if it be rendered alkaline the alkaline spectrum. If the urine is rich in the pigment shaking with acetic ether or amyl alcohol will **extract it**. If urobilin be also present take off the acetic ether extract, acidify with acetic acid and shake well with water. Urobilin passes over into the water, leaving hæmatoporphyrin.

## CHAPTER XIX

### FATS

**Estimation of Fat by Soxhlet Method.**—The dried material to be extracted, finely ground, is placed in a suitable paper extraction thimble and placed in the extraction chamber of the Soxhlet apparatus, which is then connected with the flask, previously weighed, below and the condenser above. After the addition of the appropriate amount of ether, as anhydrous as possible, extraction is carried out, preferably on an electric heater, for six to eight hours. The ether is distilled off from the flask; when the distillation is complete, **add about 1 c.c. absolute alcohol** and then heat the flask for a short period at 100° C. to dry the contents. Cool and weigh.

Another more simple method, applicable to many substances, is given on p. 303.

### **Separation of Saturated and Unsaturated Fatty Acids.**

—10 **gms.** of suet are saponified with 10 c.c. of 40 per cent. NaOH with 40 c.c. 96 per cent. alcohol added. After completion of saponification add a few drops of phenolphthalein solution and then neutralise the solution by means of 10 per cent. acetic acid. The neutralised solution is then poured into 500 c.c. of a 2 per cent. solution of neutral lead acetate contained in a litre flask. Boil, a precipitate of lead soaps forms, cool under the tap and allow precipitate to settle. Decant off as much as possible of the *clear* supernatant fluid and then wash precipitate three times with about 150 c.c. of warm water (50°–60° C.). Let the precipitate drain well and finally remove last traces of water by means of filter paper. Place the dried lead soaps in a flask, add 150 c.c. ether and heat under a reflux condenser for ten to fifteen minutes in a boiling waterbath. Allow to cool and stand overnight. Filter ethereal solution and add to the filtrate dilute HCl (1 : 4) until a precipitate forms (usually requires about 60 c.c. acid). The ethereal extract contains the lead salts of

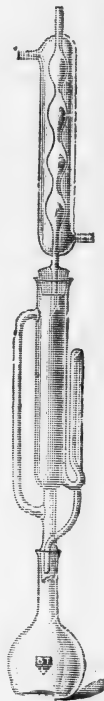


FIG. 201.

the unsaturated fatty acids, as these are soluble in ether, whereas the precipitate consists of the soaps of the solid fatty acids.

### Fat Values

I. **Melting Point.**—Use ordinary method, see p. 209.

#### II. Specific Gravity.

EXPERIMENT. Melt small amounts of butter and of oleomargarine and drop the melted fats into alcohol at room temperature (15° C.). The butter will sink, but oleomargarine will float since it is composed of fats of lower specific gravity than those of butter.

III. **Acid value** indicates the amount of free fatty acid which is present in the fat. The value rises when fats become rancid.

EXPERIMENT. Dissolve 1 gm. of fat (butter) in as little neutral alcohol as possible (with the addition of ether, if necessary), and, after adding a few drops of phenolphthalein titrate with  $\frac{N}{10}$  KOH. The result is expressed as the number of milligrams of KOH required to neutralise the fatty acid of 1 gm. of fat. In the subjoined table the result is calculated as oleic acid on the basis that 1 c.c.  $\frac{N}{10}$  KOH = 0.0282 gm. oleic acid.

IV. **The Saponification Value.**—This is a measure of the total amount of fatty acid (both free and combined) contained in the fat. The fat is saponified with a known amount of caustic potash which is in excess of that required to produce complete saponification, and the caustic potash which is not neutralised in the process is ascertained by titration against standard acid.

EXPERIMENT. Weigh a dry, clean, wide-mouthed Erlenmeyer flask, and add to it 2 gms. of melted and filtered fat. By means of a pipette add exactly 25 c.c. alcoholic potash, a sample of which has just previously been titrated against  $\frac{N}{2}$  HCl, using phenolphthalein as indicator. Close the flask with a cork having a wide glass tube passing through it. This serves as a reflux condenser. Place the flask on a boiling waterbath for half an hour, and shake frequently. Then remove the flask, add 1 c.c. phenolphthalein solution and titrate against  $\frac{N}{2}$  HCl. The difference between the amount of acid now required and the amount of acid corresponding to 25 c.c. of the alcoholic potash, as determined by the previous titration, corresponds to the amount of fatty acids. The result is usually calculated in terms of the number of milligrams of KOH required to saponify 1 gm. fat. 1 c.c.  $\frac{N}{2}$  KOH contains 0.028 gm. KOH.

V. **The Ester (ether) value** represents the amount of fatty acid which is combined with glycerine. It is obtained by deducting the acid value (III) from the saponification value (IV).

VI. **The Iodine value** is the percentage amount of iodine which a weighed quantity of fat can absorb. This is proportional to the amount of unsaturated fatty acid (oleic, etc.) in the fat (see p. 222). The iodine value is of great importance in physiological investigations, since by it we can form an estimate of the relative amount of unsaturated fatty

The following table gives some of the above values for fats of greatest physiological importance:—

Name of Fat.	Specific Gravity at 15° C.	Melting Point °C.	Acid Value expressed as per cent. Oleic Acid.	Saponification Value.	Iodine Value.	Acetyl Value of the Fatty Acid. <sup>1</sup>	Reichert-Meißl Value.
Butter . . .	0.926-0.940	28-33	0.84-2.4	220-245	26-38	9.6-18.2	25-32.8
Mutton . . .	0.937-0.961	44-51	0.72-9.3	195.2-196.5	32.7-46.2	—	—
Dog . . .	0.923	37-40	0.7-1.5	194.4-196.4	58.5	9.5-12.3	0.5-0.63
Cat . . .	0.9304	39-40	1.15-12.8	190.7	54.5	10	0.9
Horse . . .	0.919-0.933	20-42	0.87-1.22	195.1-199.5	78.8-94	6.6-14	0.44-2.14
Ox. . .	0.931-0.938	42-49	0.4-0.5	195.8-198.1	39.2-51	—	1.1
Pig . . .	0.931-0.938	36-46	0.2-2.0	193-200	50-70	—	—
Rabbit . . .	0.9345-0.9435	35-38	2.35-4.85	198.3-200.3	96.9-102.8	41.7	—

<sup>1</sup> The acetyl value indicates how much "hydroxyl" the fatty acid contains.

acids in fats. Its determination involves the use of carefully standardised solutions, and is too complicated for description here.

VII. **The Reichert-Meissl value** indicates the amount of volatile soluble fatty acids present. It is of great value in testing the purity of butter, because this contains a considerable proportion of such acids, whereas the cheaper fats, which are sometimes used as substitutes for butter, do not contain much of them.

**EXPERIMENT.** 5 gms. melted fat is saponified with alcoholic potash, the alcohol evaporated, and the resulting soap dissolved in water acidified with sulphuric acid, and distilled. The distillate, which contains the volatile acids, is collected in a flask and titrated with  $\frac{N}{10}$  NaOH, the result being expressed as the number of c.c. of decinormal acid contained in the distillate from five gms. of fatty substance.

VIII. **The Hehner value** indicates the amount of non-volatile and insoluble fatty acids (and unsaponifiable matter) present in the fat.

IX. **The Acetyl value** gives the amount of KOH which is required to combine with the acetic acid in 1 gm. of fat previously acetylated, i.e. the hydroxyl value.

## CHAPTER XX

### THE FÆCES

These are composed of:—

- (1) Substances which have escaped digestion, e.g. muscle fibres, elastic tissue, cellulose, fat, etc.
- (2) Remains of gastro-intestinal secretions.
- (3) Products of digestion by ferments or bacteria.
- (4) Micro-organisms. These often form quite a marked proportion of the faecal mass. It has been said that as much as a half of the total nitrogen in the faeces may be of bacterial origin.

*Amount.*—The daily faecal excretion of an adult male upon an ordinary mixed diet will average from 100 to 170 gms. with a solid content of between 25 and 45 gms. The amount of faeces passed when the subject is on a vegetable diet is much greater, may even amount to 400 gms. or more. The water content varies within fairly wide limits. The colour depends largely upon the nature of the diet, a meat diet, for instance, giving a dark brown colour and a milk diet a light-coloured stool. The colour may be altered by the taking of drugs such as iron, calomel or bismuth.

*Reaction.*—Normal stools have, as a general rule, a neutral reaction although slightly alkaline or slightly acid faeces are frequently met with, the alkaline reaction being more common than the acid one.

*Nitrogenous Substances.*—Faeces always contains a certain amount of nitrogenous matter, from 0.5 to 2 gms. per diem. Most of this probably comes from waste material from the various digestive secretions, micro-organisms, etc. The proteins in vegetable food-stuffs give rise to a greater amount of nitrogen in the faeces than do proteins of animal origin, since digestion is often less perfect.

*Lipoid Substances.*—Faeces always contain under perfectly normal conditions a fair amount of lipid material, neutral fats, free and

combined fatty acids, etc., even amounting to about one-third of the total dry weight of the fæces.

**Carbohydrates.**—On hydrolysis fæces normally yield reducing substances equivalent to from 0.5 to 2 gms. of glucose. Under certain conditions the output of carbohydrate may be markedly increased.

Where it is desired to get the fæces belonging to a definite period of feeding a fairly accurate separation may be got by giving the patient 5 gm. of charcoal at the start and at the end of the feeding period.

**Quantitative Analyses.**—Except for the water content most of the analyses may be carried out on fæces which has been dried on the waterbath and then ground to a fine powder.

**Estimation of the Water Content and Ash.**—(a) Of the thoroughly mixed fæces take about 3 gms. on a small watch glass, weigh exactly, shake the fæces into a small weighed platinum crucible (a silica crucible will serve), and again weigh the watch glass. The difference between the two weighings gives the amount of fæces actually used. Dry the fæces in the crucible at 110° in a hot-air oven to constant weight. The difference between the dry and the wet weight gives the water content.

(b) To find the amount of ash. Heat the crucible, at first gently, and then complete the ashing with a strong flame. Cool and weigh.

**Estimation of Nitrogen by Kjeldahl Method.**—Take 2–3 gms. of moist fæces weighed as above or about 0.5 gm. of the dried fæces and place in a combustion flask with a small piece of copper foil and 20 c.c. N-free  $H_2SO_4$  and heat till the solution is colourless (for details see Analysis of Urine). A globule of mercury may have to be added instead of the copper as fæces, particularly if they contain much fat, are somewhat difficult to combust completely. If mercury be used, before distillation some sodium sulphide or hyposulphite must be added in order to break up certain Hg.-N. compounds which are formed during combustion.

**Estimation of Fat.**—This may either be done by using the Soxhlet extraction apparatus or by grinding up the fæces with ether.

(a) Soxhlet method. Take about 5 gms. dried fæces and extract for six to eight hours in a Soxhlet apparatus with dry ether. Distil off the ether in the previously weighed small Soxhlet flask. When the distillation is finished add about 1 c.c. absolute alcohol to the flask and dry at 100° C. Cool and weigh. The increase in weight of the flask gives total extractable fatty substances.

(b) A quicker method of estimating the fat content.

(1) Take 1 gm. dried fæces, place in a mortar, rub up with 30 c.c. ether, allow to settle, then decant ether through a fluted filter paper into a weighed beaker or flask. Repeat this grinding process three times. Wash the filter paper, remembering the edges, thoroughly with ether. Distil off the ether in the flask on a hot plate or waterbath. When distillation is completed add 1 c.c. absolute alcohol to flask and dry at 100° C. Cool and weigh. Increase in weight of flask gives total fat and free fatty acids.

(2) Now add to the flask containing the total fat and free fatty acids 50 c.c. of pure alcohol previously rendered slightly alkaline to phenolphthalein by titration with  $\frac{N}{10}$  NaOH. Heat carefully until all the fat and fatty acids are completely dissolved, cool and titrate with  $\frac{N}{10}$  NaOH; add a few drops more phenolphthalein as indicator if necessary. This

gives amount of free fatty acids present. Calculate the oleic acid by multiplying number of c.c.  $\frac{N}{10}$  NaOH required by 0.0282.

(3) Brush off the residue in the mortar and on the filter paper into a beaker. In order to get every trace of material from the filter paper to the beaker pierce the bottom of the filter, wash once with boiling water, then moisten it with a few drops of concentrated HCl. Wash repeatedly with boiling water until every trace is in beaker. Add HCl up to 10 per cent. Place beaker on a boiling water bath for two hours. Cool. Pour into a separating funnel and extract three times with ether. Wash the ethereal extract three times with small amounts of water. Place ethereal extract in a weighed flask, distil off the ether, dry and weigh. Result = combined fatty acids.

**Estimation of Carbohydrate.**—Take 20 gms. of dried faeces and place it in an Erlenmeyer flask (300 c.c. capacity) with 100 c.c. 2 per cent. HCl, connect with a reflux condenser and boil for one and a half to two hours. Almost neutralise and then filter into a litre flask, washing the residue on the filter paper thoroughly, and make up to 1 litre. The sugar present may either be estimated by a titration method or preferably by a gravimetric method. Place 60 c.c. of freshly mixed Fehling solution in a small Erlenmeyer flask and immerse the flask in a boiling waterbath. When the Fehling solution has attained the temperature of the boiling water add 20 c.c. (which should not contain more than 0.25 gm. reducing sugar, check by a rough analysis beforehand) of the solution to be tested, previously warmed, and make contents of flask up to 100 c.c. by the addition of boiling distilled water. The mouth of the flask is lightly plugged with cotton wool and the heating continued for exactly twelve minutes. At the end of the period the flask is removed from the bath and the contents filtered through asbestos<sup>1</sup> in a Gooch crucible. Wash well with hot water, taking care that all traces of cuprous oxide are transferred from the flask to the crucible, then with alcohol and ether. Dry quickly in a hot-air oven, cool and weigh as cuprous oxide. If the Gooch crucible containing the cuprous oxide be placed in a protecting nickel crucible and heated by means of a powerful burner for thirty minutes the cuprous oxide is oxidised to cupric oxide. Continue heating until weight is constant. Cool and weigh. The cuprous oxide can also be, if preferred, reduced to metallic copper by gentle heating in a stream of hydrogen. Cool and weigh.

Calculation of results—Weight of precipitate as

$$\left. \begin{array}{l} \text{Copper} \times \cdot 5638 \\ \text{Cuprous oxide} \times \cdot 5042 \\ \text{Cupric oxide} \times \cdot 4535 \end{array} \right\} = \text{amount of sugar (as dextrose) present.}$$

**Estimation of Calcium.**—Take 5 gms. of the faeces and ash it in a small silica crucible (if platinum is not available). Transfer the ash to a 500 c.c. measuring flask, washing in the last traces with dilute HCl. When the ash in the flask has all dissolved in the HCl make up with distilled water to the 500 c.c. mark. Draw off 200 c.c. into a 600 c.c. beaker and add a *slight* excess of ammonia until a precipitate of calcium phosphate appears, then add acetic acid until the solution is faintly acid

<sup>1</sup> The asbestos used in preparing the mat in the Gooch crucible should be specially purified by treatment with hydrochloric acid. All hard lumps should be removed. The same crucible can be used for many analyses. The mat should be about 2 mm. thick.



and the phosphate precipitate has redissolved. If there has been any iron in the ash, and faeces commonly contains small amounts of iron, a small precipitate of iron phosphate may remain after the treatment with acetic acid. This precipitate must, if it be present, be filtered off. Heat the clear solution to boiling point, add excess of ammonium oxalate (20 per cent. solution) until the precipitation of lime is complete. Allow to settle completely (best by standing overnight). Filter off precipitate through a 12 cm. Swedish filter paper, washing thoroughly with boiling water. The calcium may be estimated either gravimetrically (a) or by titration (b).

(a) Ignite the dried filter paper and precipitate in a platinum or silica capsule over a blowpipe until constant in weight. Weigh as CaO.

(b) Pierce the damp filter paper on the funnel with a glass rod and wash through the precipitate into the beaker originally used for the precipitation with water. Next, in order to remove the last traces of the precipitate from the paper, wash repeatedly with small quantities of 1 in 10  $H_2SO_4$ . Heat the beaker to about  $75^\circ C.$  and continue heating till the calcium oxalate is all dissolved. Titrate at once with  $\frac{N}{10}$  potassium permanganate and calculate to CaO.

1 c.c.  $\frac{N}{10}$  permanganate solution = 0.0028 gm. CaO.

**Tests for "Occult" Blood.**—These tests are only of value when a meat-free diet is taken. The benzdine and phenolphthalin tests are very delicate when properly applied. The guaiac test although not so sensitive is quite useful.

(a) *Guaiac Test.*—Stir up 5–10 gms. faeces in a mortar with water until it forms a thick fluid. (If the faeces is rich in fat treatment with ether should first be carried out.) Add one-third of its volume of glacial acetic acid and mix thoroughly. Pour about 10 c.c. into a test tube, add an equal volume of ether and mix thoroughly. Allow ether to separate (aid of a centrifuge is useful), collect in a test tube, add 10 drops of about 1 per cent. guaiac solution and about 4 c.c. of 10 per cent. hydrogen peroxide. The whole is shaken. Provided no pus has been present the blue colour may be assumed to indicate blood.

(b) *Benzidine Test.*—Dissolve a knife point full of pure benzidine (keep in a dark place) in 2–3 c.c. glacial acetic acid and add 2 c.c. hydrogen peroxide. A small quantity of faeces is shaken up with a little hot water and 2–3 c.c. added to the benzidine solution. If blood be present a bluish or greenish colour develops in two or three minutes. Delicacy is about 1 : 100,000.

(c) *Phenolphthalin Test.*—To a suspension of a small amount of faeces in water (about 2 c.c.) add about 1 c.c. of the phenolphthalin reagent. (Add 2 gms. phenolphthalein and 10 gms. of zinc dust to 100 c.c. 20 per cent. caustic soda. Heat the bright red solution carefully until it has become slightly yellow in colour, i.e. until the phenolphthalein is reduced to phenolphthalin. Pour off supernatant fluid and keep in the dark, adding a little liquid paraffin to prevent surface oxidation.) Then add 1, at most 2, drops of 10 per cent. hydrogen peroxide. A bright red colour will develop, due to reoxidation, if blood be present. Delicacy is about 1 : 800,000.

## CHAPTER XXI

## DIGESTIVE SECRETIONS

**Estimation of Diastatic Activity.**—For the accurate determination of the action of ptyalin (or any other amylolytic ferment) on starch one may estimate the reducing power of the incubated solution after a certain time. Besides being tedious, this method is uncertain, because of the different reducing powers of maltose and dextrose, both of which sugars frequently result by salivary digestion, especially when this is prolonged.

A simpler and more serviceable method depends on the colour reaction of starch with iodine, and is conducted as follows:—

Prepare a 1 per cent. starch paste solution,<sup>1</sup> and place the beaker containing it in ice water. Collect some saliva and dilute 1 c.c. of it to 10 c.c. with distilled water, and filter. Take a series of five test tubes labelled A, B, C, etc., and with a 1 c.c. pipette graduated in 100 parts deliver into tube A 1 c.c. of the diluted saliva; into B 0.75 c.c.; into C 0.5; into D 0.25; and into E 0.1.

Place the tubes in a beaker containing ice water, and then deliver into each 5 c.c. of 1 per cent. cooled starch solution. The cold prevents any ferment action until all are ready. Now remove the tubes to another beaker containing water at 40° C., and gently shake them so that the contents become thoroughly mixed. Note the exact time at which the tubes are placed in the warm water. At the end of half an hour remove the tubes simultaneously to ice water, and shake them gently so as to ensure thorough cooling. Fill each tube to within half an inch of the top with distilled water and add a few drops of iodine solution.<sup>2</sup> Close each tube with the finger and invert so as to mix. It will be seen that there is a gradation of colours in the different tubes from blue through violet and brown to yellow. Note the tube which just shows a bluish tint. The next one higher up in the series is taken as that in which all starch has just disappeared. From the amount of diluted solution added to this, calculate the amount of undiluted saliva required to convert 100 c.c. of 1 per cent. starch solution into dextrines in half an hour at 40° C. Thus, suppose that the tube containing 0.25 c.c. diluted saliva is found to be that which just shows a bluish tint. In the next (*viz.* containing 0.5 c.c. saliva)

all the starch has disappeared, therefore  $\frac{0.5}{10} = .05$  c.c. saliva can hydrolyse 5 c.c. 1 per cent. starch, or 1 c.c. can invert 100 c.c. 1 per cent. starch. The diastatic action of pancreatic juice, of liver extract, of blood serum or of malt diastase may be measured in the same way, but different amounts of the ferment solution must be employed.<sup>3</sup>

<sup>1</sup> Weigh 1 or 2 gms. of pulverised "soluble starch," and stir it up in a beaker with an amount of distilled water sufficient to make a 1 per cent. solution. Place on a boiling waterbath and continue stirring until a clear opalescent solution is obtained. Cool before using.

<sup>2</sup> Care must be taken that sufficient iodine solution is added to give the maximal reaction, but an excess must be avoided. The iodine solution is made by dissolving 12.7 gr. iodine in water containing 25 gr. potassium iodide, and then diluting to 1,000 c.c.

<sup>3</sup> Care must be taken when using organ extracts, such as those of liver,

(Thus for blood serum and liver extract it is unnecessary to dilute the solution.) The results may be expressed by the formula  $^1 D_{\frac{40^\circ}{30}}$ , in which the temperature and the length of time of incubation are shown. In the above example  $D_{\frac{40^\circ}{30}} = 1$ .

To study the influence of weak acids, etc., on the action of ptyalin the above method is very satisfactory, i.e. by adding some acid solution to one or more of the tubes. In some cases it is desirable to prolong the incubation for twenty-four hours, in which case some chloroform or toluol or thymol should be added to retard the development of micro-organisms. If very close results are desired, the observation should be performed with amounts of ferment solution which vary from one another by smaller amounts, or a second observation should be made taking amounts of ferment solution lying between the faintest blue and the next tube.

It is of interest to compare by the above method the comparative diastatic powers of the various commercial preparations of diastase, taking human saliva as the standard.

**Preparation of Extract of Gastric Mucosa.**—Scrape off the mucosa of a well-washed stomach of a pig, mix the scrapings with 100 times their bulk of 0.4 per cent. hydrochloric acid and digest the mixture for several hours at  $40^\circ \text{C}$ . Filter the extract through muslin and use for digest experiments. If a better extract be required excess proteoses may be got rid of by allowing digestion at  $40^\circ$  (in incubator) to proceed for several days. The extract is then saturated with ammonium sulphate and the resulting precipitate of proteoses, which also contains the pepsin, pressed free of fluid, is again incubated for several days after dilution with several volumes of 0.5 per cent. hydrochloric acid. The precipitate formed after saturation of this digest with ammonium sulphate is rich in pepsin. Excess ammonium sulphate may be got rid of by dissolving the precipitate in water and dialysing it.

An active extract may also be obtained by treating the mucosa scrapings with dilute hydrochloric and then extracting for some days with glycerine.

**Demonstration of Pepsinogen.**—Grind up scrapings of about 3 square inches gastric mucosa with some sand in a mortar and add gradually 20 c.c. of 1 per cent. sodium carbonate solution. Filter. The filtrate will not digest fibrin. Add then dilute HCl drop by drop until the filtrate is faintly acid to litmus and again incubate with fibrin. Digestion takes place. Divide this acid filtrate into two parts. To one add 1 per cent. sodium carbonate solution until alkaline and place at  $40^\circ \text{C}$ . for fifteen minutes after which again render it faintly acid with HCl. To both tubes add a piece of fibrin and digest in usual way. Digestion occurs in tube which has been kept acid in reaction but not in the other. Dilute alkali has no effect on pepsinogen, but it destroys pepsin.

**Methods of Estimating Activity of Pepsin Solutions.**—Grützner's method, where the fibrin used was stained by means of carmine, was rendered more useful by Roaf, who used congo red as the indicator.

that the reaction of the incubation mixture is kept constant. This is best accomplished by adding a few drops of a saturated solution of  $\text{Na}_2\text{HPO}_4$  to the solutions.

<sup>1</sup> D = diastatic power.

This method, when congo red is used, serves for the rough estimation of both gastric and pancreatic juices. Place an equal amount (weighed) of stained fibrin<sup>1</sup> into two test tubes, and according to the ferment under investigation add 15 c.c. 0.4 per cent. HCl or 0.4 per cent. Na<sub>2</sub>CO<sub>3</sub> solution to one, and to the other 10 c.c. 0.4 per cent. HCl or 0.4 per cent. Na<sub>2</sub>CO<sub>3</sub> solution and 5 c.c. of the gastric or pancreatic juice to be tested. Mix well and place in a water bath or incubator at 40° C. for fifteen to twenty minutes. Filter off undigested fibrin and examine the filtrate. Or if preferred two samples of digestive juice of different strengths may be compared. The more active the juice the more pigment in solution. The colour in the case of gastric digestion is bluish and is difficult to compare; the red colour can be restored if the solution is rendered just alkaline by the addition of a very small amount of solid sodium carbonate.

Other methods have been introduced for the quantitative estimation of both pepsin and trypsin in which the amount of digestion of proteins like edestin, ricin, egg albumen and caseinogen is determined. The test with caseinogen is perhaps most readily carried out as the material required is most easily obtained; 1 gm. of commercial casein is dissolved in 16 c.c. of 25 per cent. HCl (sp. gr. 1.124) in a litre flask on the waterbath and then made up to 1,000 c.c. with water; 10 c.c. of this solution are placed in each of a series of test tubes and then varying amounts from 0.1 to 1 c.c. of the gastric juice to be tested are added. The tubes are then placed in a waterbath at 40° C. and allowed to digest for fifteen minutes. To each test tube there is then added a few drops of a 20 per cent. solution of sodium acetate; this precipitates all undigested caseinogen. The first tube in which a mere trace of clouding is observed is taken as containing the amount of enzyme which just sufficed to complete the digestion. The result is usually stated in terms of units. The reciprocal of the amount which just suffices to complete digestion gives a measure of the proteolytic activity of the juice, e.g. in a test if 0.03 c.c. of juice sufficed to complete the digestion the value would be put as  $\frac{1}{.03}$  or 33 units.

When trypsin is to be determined the caseinogen (0.1 gm.) is dissolved in 5 c.c.  $\frac{N}{10}$  NaOH, 25 c.c. water and then solution is boiled. After cooling the alkaline solution is neutralised with  $\frac{N}{10}$  HCl and the volume made up to 100 c.c. (This solution does not keep well and ought always to be freshly prepared.) A series of test tubes, as before, are filled with 2 c.c. caseinogen solution, varying volumes of trypsin solution or pancreatic juice added. At the end of the period of digestion (one hour) caseinogen not digested is precipitated by means of acid alcohol (1 c.c. glacial acetic acid, 49 c.c. water and 50 c.c. 95 per cent. alcohol). End point and calculation as above.

**Mett's Method.**—A narrow glass tube (1–2 mm. diam.) drawn to a

<sup>1</sup> Carefully cleaned minced fibrin is allowed to soak in 0.5 per cent. congo red solution for twenty-four hours (50 gms. moist fibrin per 100 c.c. solution). It is then poured into a large volume of water and heated for five minutes to 80° C. It is then collected on a cloth, well washed under the tap, squeezed as dry as possible and kept in equal parts glycerine and water. Add a little toluol as preservative.

fine point at both ends, is filled with egg white, the ends closed in the flame, and the tubes then heated in a boiling waterbath so that a column of coagulated albumin is obtained. It is then cut into segments of equal length, and two of these are placed in a test tube or Petri dish which contains the pepsin solution, acidified with 0.2 per cent. hydrochloric acid. Two similar tubes are placed in another test tube or Petri dish with another pepsin solution of different strength. Both are placed in the incubator for several (ten) hours. The length of dissolved protein column is then measured in both cases, and the desired result is obtained by squaring this distance.

Thus if in one test tube the length were 2, and in the other 3, the strength of the two pepsin solutions has the ratio of 4 to 9.

This method is only accurate when weak pepsin solutions are used. If more than 4 mm. of protein are digested, the estimation must be repeated with diluted solutions.

**Isolation of Amino Acids.**—Mince up a pig's pancreas thoroughly, and shake it in a flask with 500 c.c. of water containing 3 c.c. of a saturated solution of sodium carbonate, and 3 c.c. of chloroform. Add also about 200 gms. of blood fibrin, which has previously been soaked in 1 per cent. sodium carbonate solution. Place the flask in an incubator at body temperature, and after three days test the reaction of the digest towards litmus. If acid, add more sodium carbonate till distinctly alkaline. Also remove about 10 c.c. and filter into a test tube. To this sample carefully add a few drops of bromine water. A violet colour results, the intensity of which should be carefully noted. This colour reaction is due to tryptophan, an aromatic amino acid which is liberated by the action of trypsin.

Test the reaction towards litmus and the intensity of the tryptophan reaction on each succeeding day. When the tryptophan reaction becomes very intense (in about five days) proceed to isolate leucine and tyrosine in the following manner:—

The digest is rendered faintly acid with acetic acid, boiled and filtered hot. A sample of the filtrate is removed and tested for proteose. A negative result is usually obtained.

**1. Separation of Tyrosine.**—The remainder is evaporated on the waterbath to a thin syrup. This is allowed to stand on ice or in a cold place for several days. White flocculi of *tyrosine* separate out. These are filtered through fine muslin, and removed to a beaker by means of a jet of cold distilled water and washed several times with distilled water by decantation. They are then dissolved by boiling with water made alkaline by the addition of a few drops of ammonia, and the resulting solution is quickly filtered hot. The filtrate is heated till all the ammonia is expelled; it is then cooled, when the tyrosine separates out as a white precipitate. This is collected on a filter paper, washed, and dried. The following reactions may be applied to the resulting powder:

(1) Tyrosine is insoluble in cold water, slightly soluble in hot water, and very soluble in dilute alkali.

(2) A solution in hot water gives a red colour on the addition of Millon's reagent. This is because tyrosine contains an aromatic radicle (p. 196).

(3) *Piria's Test.*—Place some of the powder in a dried test tube, add about 2 c.c. concentrated sulphuric acid, and place the test tube in a boiling waterbath for half an hour. Now cool and dilute with water, transfer to an evaporating basin, and remove the sulphuric acid by

adding powdered barium carbonate; filter off the barium sulphate, evaporate the filtrate to small bulk, and add a drop or two of very weak ferric chloride solution. A violet colour results.

**2. Separation of Leucine.**—The tyrosine-free filtrate is evaporated till a skin of leucine forms on the surface. It is then mixed while still warm with several times its bulk of alcohol, whereby a precipitate (previously known as antipeptone) separates out, which after cooling can be removed by filtration. This precipitate consists of a mixture of several bodies, including lysine, histidine, and arginine. The filtrate is evaporated on the waterbath until all the alcohol has been driven off. It is then boiled with lead carbonate and filtered. The lead is removed from the filtrate by means of  $H_2S$ , the  $PbS$  separated by filtration, and the final filtrate accurately neutralised with weak  $NaOH$ . By now concentrating by evaporation on the waterbath and cooling leucine will separate out.

*Reactions of Leucine.*—(1) It is much more soluble in water than is tyrosine; it is soluble also in alcohol.

(2) When heated in a piece of dry glass tubing, a sublimate forms on the cool parts of the tube.

(3) Like other amino acids, it gives off ammonia gas when heated in a test tube with a piece of solid caustic potash and a few drops of water. If the melt be cooled, dissolved in water, and then acidified with sulphuric acid, it gives a smell of valerianic acid on heating.

(4) *Scherer's Test.*—Heat some leucine with a drop of nitric acid on a piece of platinum foil, add to the dry residue some caustic potash, when a yellow stain results. Heat still further, and the stain rises up into a globule which runs off the platinum.

(5) Examine a solution of leucine with the polariscope (p. 212). It is *lævo-rotatory* ( $a_D$ )—in aqueous solution =  $-10.8^\circ$ . The leucine which is obtained by boiling protein with baryta, or that obtained synthetically is *optically inactive*, and the *dextro-rotatory* form may be obtained from this by allowing penicillium glaucum (a fungus) to grow on a solution of it. The fungus destroys the *lævo-rotatory* part, but leaves the *dextro-rotatory* untouched. Moulds, yeasts and ferments act much more energetically on naturally occurring than on synthetic isomers.

**Tryptophan.**<sup>1</sup>—If bromine water be cautiously added to a tryptic digest of several days' standing a deep violet-red colour will result, and if the mixture be shaken with amyl alcohol, this latter will take up the colour. The glyoxylic reaction (see p. 196) will also be very distinct in the digest even after the Biuret reaction has disappeared (i.e. after the protein molecule has been quite destroyed). Both these reactions are due to tryptophan, which is closely related in its chemical structure to certain of the aromatic substances that are produced by the bacterial digestion of protein.

**Separation of Tryptophan.**—A large amount (500 gr.) of commercial casein is mixed with liquor pancreaticus (200 c.c. Benger) and 0.8 per cent.  $Na_2CO_3$ , and placed in an incubator for about a week. The ferment should be added, half at the beginning and the remainder three or four days later. Antiseptics should be added.

<sup>1</sup> A single digestion mixture may be employed for the separation of leucine, tyrosine and tryptophan, but in such a case both fibrin and casein ought to be added, since casein is the only common protein which yields any large amount of tryptophan. It also contains a considerable amount of tyrosine.

Digestion is allowed to proceed until the bromine water reaction is maximal. The digest is then boiled, cooled and filtered, and  $\text{H}_2\text{SO}_4$  added to the filtrate, so as to bring the amount of  $\text{H}_2\text{SO}_4$  in the latter to 5-6 per cent. If any precipitate is hereby formed it should be filtered off. The clear filtrate is then mixed with an excess of an acid solution of mercuric sulphate (10 per cent. mercuric sulphate dissolved in 10 per cent.  $\text{H}_2\text{SO}_4$ ) and filtered. This reagent may precipitate, besides tryptophan, some tyrosine and cystine.

From tyrosine the precipitate is freed by washing it with 5-6 per cent.  $\text{H}_2\text{SO}_4$ , the mercury compound of tyrosine being very soluble in this. From cystine (which is scanty in a digest of casein) the tryptophan is separated by reprecipitation. For this purpose the washed mercury precipitate is suspended in water and decomposed with  $\text{H}_2\text{S}$  gas. To complete this reaction the suspension must be saturated with the gas, then warmed and saturated again. The  $\text{HgS}$  precipitate is filtered off, the filtrate warmed to rid it of  $\text{H}_2\text{S}$ , then acidified to 5-6 per cent.  $\text{H}_2\text{SO}_4$ , and the mercuric sulphate reagent added to it until a small permanent precipitate is produced. This is mainly cystine, and is filtered off. The tryptophan in the filtrate is then completely precipitated by mercuric sulphate, and the resulting precipitate treated exactly like the first one.

In this way a solution of tryptophan in 5-6 per cent.  $\text{H}_2\text{SO}_4$  is obtained. The  $\text{H}_2\text{SO}_4$  is now precipitated by adding  $\text{Ba}(\text{OH})_2$  water in the heat and filtering. Great care should be taken that the filtrate contains no excess either of  $\text{H}_2\text{SO}_4$  or of  $\text{Ba}(\text{OH})_2$ . The watery solution of tryptophan is then mixed with half its bulk of alcohol and evaporated on a waterbath. During evaporation small quantities of alcohol are added from time to time to prevent the browning which occurs if watery solutions of tryptophan are heated alone. Evaporation proceeds till crystallisation commences, when the basin is removed and allowed to stand. The crystals (glistening plates) are collected on a filter, and, to purify them, may be recrystallised.

A solution of the crystals gives the bromine and the glyoxylic reactions very distinctly, and if the crystals be heated in a test tube indol and scatol (see p. 238) are evolved.

Tryptophan is the mother substance of indol, which, along with its methyl derivative scatol, is largely responsible for the faecal colour. These bodies are produced from tryptophan by bacterial growth (see p. 238).

**Preparation of Cystine** (Folin).—Place 50 gms. wool in a 500 c.c. flask, add 100 c.c. conc.  $\text{HCl}$  and place on a waterbath until all dissolved. In order to prevent undue loss of fluid close mouth of flask with a cork carrying a 3-foot length of glass tubing to act as a simple condenser. When completely dissolved boil *very gently* over a small flame for three to four hours. Add solid sodium acetate (100-130 gms.) until congo red paper no longer turns blue. Allow mixture to stand three to five days (the longer the better up to three weeks), then filter on a Buchner funnel and wash with cold water. Dissolve precipitate in 150 c.c. water + 5-10 c.c. conc.  $\text{HCl}$ , add about 20 gms. purified bone charcoal and boil 5-10 minutes. Filter again with suction, heat filtrate to boiling, neutralise hot by adding very slowly hot concentrated sodium acetate solution, taking care not to add an excess (test constantly with congo red paper). Precipitate formed is cystine. If crystals not white repeat solution and treatment with charcoal. Note crystal form and

test for sulphur. A fair yield of tyrosine may be had from the original mother liquor.

Preparations of trypsin have a rennin-like action on milk if sufficient calcium be added (see p. 232).

**Intestinal Juice. Succus Entericus.**—This is secreted by Lieberkuhn's follicles.

Extracts of the mucous membrane of the intestine, prepared by scraping this off and grinding it with sand and water and then filtering through muslin, usually contain large amounts of ferments. This extract will contain both exoenzymes and endoenzymes.

**To Demonstrate the Ereptic Power of Tissues.**—Take 20 gms. minced liver, and 20 gms. mucous membrane of the intestine (scraped off with a scalpel). Grind each in a mortar with fine quartz sand and 20 c.c. of a 0.2 per cent. solution of  $\text{Na}_2\text{CO}_3$ . Filter the extracts through muslin. Divide each extract into two equal parts, A and B. To A of each extract add 1 c.c. of a 2.5 per cent. solution of Witte's peptone, and to B a similar amount of a 2.5 per cent. solution of egg-white. Remove a few drops of the contents of each of the four test tubes, and apply the Biuret test, noting the results. Place the tubes in the incubator at body temperature, and at the end of an hour again remove a little of the contents of each tube, and apply the Biuret test. It will be found that there is no change in the tube (B) containing egg-white, but that in the tube (A), containing the intestinal extract, the test has become very feeble or disappeared entirely. By longer incubation, the Biuret secretion will also disappear from the tube (A) containing liver.

By thus ascertaining the time required to split up a standard solution of peptone, so that the Biuret test is no longer given, a comparative estimate may be made of the ereptic power of different extracts.

**Separation of Bile Salts.**—Thoroughly mix 50 gms. pure animal charcoal with 200 c.c. of ox-bile in an evaporating dish, and evaporate the mixture to dryness on a waterbath. During the drying the mixture should be frequently stirred. The black powder thus obtained can be kept a considerable time. To extract the bile salts from it, mix it with *absolute* alcohol in a flask and place the flask on the boiling waterbath for about a quarter of an hour, cool, filter into a dry cylinder, and add anhydrous ether to the filtrate till a permanent haze is produced. Now cover the cylinder with a ground glass plate, and allow it to stand in a cool place till next day, when it will be found that a crystalline mass of bile salts has separated out (Plattner's Crystalline Bile). The crystals can now be collected on a filter paper and allowed to dry in a desiccator.

A 1 per cent. solution of the crystals should now be made, and Pettenkofer's reaction (see p. 236) applied to it by the following method:

Dissolve a few grains of cane-sugar in the solution, and run concentrated sulphuric acid down the side of the tube so as to form a layer underneath the watery solution. A violet ring is formed where the two fluids meet. Now place the test tube in a beaker of cold water, and shake gently so as to mix the two fluids. A violet solution is thus obtained. (By cooling the test tube in water too great a rise of temperature is avoided.) Divide the violet solution into two parts, A and B. Add A to some ether and examine by means of the spectroscope—a distinct band is seen in the green. Add B to some absolute alcohol and note that, although the spectrum is at first the same as in A,



a band gradually develops in the blue, and that, along with the development of this, the tint of the solution changes from violet to brown.

**To Prepare Pure Glycocholic Acid.**—In certain districts of Germany and America it has been observed that the glycocholic acid can be separated from the bile by a very simple process, and, so far as it has as yet been tried, the bile obtained from oxen reared in this country appears to be suitable for the process. The method is as follows :—

Some ox bile is placed in a stoppered cylindrical vessel, and mixed with ether and hydrochloric acid in the proportion of ten parts of the former and four parts of the latter, for every hundred parts of bile. A few crystals of glycocholic acid are added to the mixture so as to start the crystallisation, the vessel is stoppered, vigorously shaken, and then allowed to stand in a cool place. After some time the mass will be found to be "solid" with crystals. These are collected in a filter paper, and washed with cold distilled water till no more pigment can be removed. They are then removed to a flask and dissolved in boiling water; the solution is filtered hot, and the filtrate, on cooling, deposits numerous acicular crystals of the acid. These may now be collected, washed with distilled water, and dried (see p. 236).

**Preparation of Taurin.**—Bile from carnivorous animals—cat or dog—is heated on a sandbath with one-third its bulk of concentrated hydrochloric acid until a resinous-like mass of the anhydride of cholalic acid (called Dyslysin) has formed. This can be drawn out into brittle threads by means of a glass rod. The dyslysin is filtered off, and the filtrate is evaporated to a small bulk, the sodium chloride, which crystallises out during the evaporation, being removed by filtration. The thin syrup is then poured into fifteen times its bulk of alcohol, and left standing twenty-four hours, when the taurin will have crystallised out. It can be purified by collecting the crystals on a filter paper, and washing with cold water.

## CHAPTER XXII

### BLOOD

**Preparation of Fibrin Ferment.**—Blood serum or defibrinated blood is mixed with twenty times its bulk of alcohol. A copious white precipitate is obtained. Allow this to stand under alcohol for six to eight weeks. All proteins other than fibrin ferment are coagulated. Decant fluid, collect the precipitate on a filter and after all alcohol has drained off grind the precipitate in a mortar with water. The watery extract which can be filtered contains the fibrin ferment.

**Estimation of the Coagulation Time** (Dale and Laidlaw).—Prepare a number of capillary tubes about 2 cm. long with an internal diameter of 1.3 to 1.4 mm. Narrow one end of a capillary tube (best to prepare a number at one time as they only serve for one estimation) in the flame, insert into the bore of the tube by the open end a leaden shot of a weight of approximately 9 mgm. Such a shot should move easily, rolling the whole length of the tube. Now narrow the open end of the capillary to prevent the shot falling out. The shot should be clean and round.

To carry out the estimation from a prick in the finger fill a capillary tube by bringing one end into contact with the drop of blood, the tube

being held inclined slightly upwards. As soon as it is filled place the ends between the jaws of a special spring clip (see *Journ. Path. and Bact.*, 16, 1911, 353) which have been prepared with clean plasticine. The clip with the tube is then immersed in water at 35°–40° contained in a white basin. The temperature must be kept approximately constant. Not more than ten seconds should elapse between the appearance of the blood and immersion of the tube in the bath. The shot is kept gently rolling from one end of the tube to the other by rotating the handle of the clip. The viscosity increases until the tube has to be held almost vertical to keep the shot travelling. Unless the tube is jerked or knocked, however, the shot should continue to travel smoothly up and down but with diminishing speed till it stops altogether with the tube held vertical. At this point the stop-watch is stopped (it was started as soon as the drop of blood appeared as the result of the prick) and the reading taken. At a temperature of 37°–38° C. with this method it was found that for normal individuals the average coagulation time was about one and three-quarter minutes.

### Quantitative Examination of some of the Various Blood Constituents

**Total Nitrogen.**—Pipette off carefully 1 c.c. of oxalated blood into an ordinary combustion flask and carry out the determination of the total nitrogen present by the ordinary Kjeldahl method as given in the analysis of urine. Or the determination may be carried out after appropriate dilution (1 c.c. oxalated blood diluted to 25 c.c. with water and 1 c.c. of this diluted blood used).

As regards the determination of the other constituents many methods have been introduced. If a series of determinations have to be carried out on a single blood the method introduced by Folin has been found to be generally useful. The main drawback is the amount of blood required. Folin's scheme will be given.

**Preparation of Protein-free Blood Filtrates.**—Collect by means of a syringe or other suitable apparatus, the blood, say 10 c.c., over finely powdered potassium oxalate, using about 20 mg. of oxalate for 10 c.c. blood. Transfer a measured quantity (5 c.c. or more) of oxalated blood to a flask having a capacity of fifteen to twenty times that of the volume of blood taken. Add seven volumes of water to luke the blood, then one volume of a pure 10 per cent. solution of sodium tungstate<sup>1</sup> ( $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ ) and mix. Add from a graduated pipette, slowly and with shaking, one volume of two-thirds normal sulphuric acid.<sup>2</sup> Close the mouth of the flask with a rubber stopper and shake well. Very few bubbles, if any, will form as the result of the shaking if the conditions are right. Stand for five minutes when the colour of the coagulum ought to change from bright red to dark brown. If this change of colour does not take place, usually due to excess of oxalate, coagulation is incomplete, but coagulation may then be ensured by the addition of 10 per cent. sulphuric acid drop by drop and vigorous shaking after each drop. Continue until practically no foaming and the change to dark brown colour occurs.

<sup>1</sup> Sodium tungstate used should be free from excess of sodium carbonate.

<sup>2</sup> Two-thirds normal  $\text{H}_2\text{SO}_4$ , 35 c.c. conc. acid diluted to 1,000 c.c. It is advisable to check by titration. Acid is used to take up the sodium from the tungstate. Tungstic acid combines with the proteins, filtrate is only slightly acid to congo red.

Now pour the mixture on to a filter large enough to hold it all. Folin advises that a few c.c. be first poured on to the double portion of the filter paper and that the remainder should be withheld until the whole filter has been wet. As soon as complete wetting takes place pour in the rest of the mixture and cover with a watch glass. If conditions are right even the first few drops of the filtrate should be water clear; no re-filtering should be necessary. If filtrate has to be kept for any time (more than two days) add a few drops of toluol as a preservative. The filtrate which results is suitable for the determination of non-protein nitrogen, urea, uric acid, creatinine, creatine and sugar.

**Determination of Non-protein Nitrogen.**—Pipette 5 c.c. of the above filtrate into a hard glass test tube (200 mm.  $\times$  25 mm.), marked at 35 and 50 c.c., which should either be perfectly dry or just previously rinsed with alcohol and drained, and add 1 c.c. diluted acid mixture<sup>1</sup> (1 part acid mixture, 1 part water). Boil vigorously over a micro burner until tube is filled with dense fumes (in from three to seven minutes, depending on the size of the flame), then reduce the flame sharply so that speed of boiling is reduced to lowest limit. Cover mouth of test tube with a watch glass and continue gentle heating for two minutes from the time the tube is filled with fumes. If solution is not then perfectly colourless heating must be continued until this end is attained. When colourless allow digestion mixture to cool for seventy to ninety seconds, then add 15–25 c.c. water, cool to room temperature and finally add more water to make total volume 35 c.c. Add 15 c.c. of Nessler's solution,<sup>2</sup> close with a clean rubber stopper and mix. If solution is turbid it must be centrifuged before doing the colour value with the standard.

Folin's standard is 0.3 mg. of N. Add 3 c.c. of standard ammonium sulphate solution (0.4716 gm. absolutely pure dry ammonium sulphate dissolved in 1 litre water, 10 c.c. of this solution contains 1 mg. of nitrogen) to a 100 c.c. volumetric flask, then 2 c.c. of the acid mixture to balance acid in other test, dilute to about 60 c.c. and add 30 c.c. of Nessler's solution. The unknown and the standard should be nesslerised simultaneously.

Calculation. If standard is set at 20 mm. in the colorimeter, 20

<sup>1</sup> *Acid Mixture.*—To 50 c.c. of a 5 per cent. copper sulphate solution add 300 c.c. of 85 per cent. phosphoric acid, mix, then add 100 c.c. concentrated N-free sulphuric acid, again mix. Keep free from all ammonia fumes.

<sup>2</sup> *Nessler's Reagent.*—Place 150 gms. KI and 110 gms. I in a 500 c.c. flask, add 100 c.c. water and an excess of metallic mercury (140–150 gms.). Shake vigorously and continuously for seven to fifteen minutes or until dissolved iodine has nearly all disappeared. Solution becomes hot. When red iodine solution begins to become definitely pale, though still red, cool in running water and continue shaking until solution assumes a greenish colour (about fifteen minutes in all). Decant off the solution from the surplus mercury, wash well with water, dilute solution and washings to 2,000 c.c.

From a completely saturated solution of NaOH (55 gms. NaOH per 100 c.c.) prepare an approximately accurate 10 per cent. solution. To 3,500 c.c. of this 10 per cent. NaOH add 750 c.c. of the double iodide solution and 750 c.c. of distilled water, giving thus in all 5 litres of Nessler's solution.

To prevent turbid mixtures on the addition of Nessler solution to the fluid to be tested as a general rule the volumetric flask or test tube should be at least two-thirds full before the addition of Nessler is made. The volume of Nessler to be added should form about 10 per cent. of the ultimate total volume of fluid and reagent. This amount is of course modified if much acid or alkali is present.

divided by the reading of the unknown and multiplied by 30 gives the non-protein nitrogen in mgs. in 100 c.c. blood.

**Determination of Urea.**—Put 5 c.c. of the tungstic blood filtrate in a clean hard glass tube (200 × 25 mm.), add 2 drops of a phosphate mixture (60 gms. monosodium phosphate and 179 gms. crystallised disodium phosphate dissolved in warm water and diluted to 1 litre) and 1 c.c. of an active urease solution. Immerse the test tube in warm water (40°–55° C.) for five minutes. In another test tube with a 25 c.c. mark place 2 c.c. of 0.05 N hydrochloric acid. After the addition to the urease tube of a dry pebble, a drop or two of paraffin and 2 c.c. saturated borax solution connect the two tubes by means of glass tubing, carrying two rubber corks and of such a length that the tubing on the receiver side dips beneath the surface of the acid. Having inserted both corks boil at a *moderately* fast, and as uniform as possible, rate for four minutes. Then slip off receiver, leaving connecting tube still inside, but free from acid solution and continue boiling for another minute. Rinse the end of the delivery tube with a little water, cool the distillate under the tap and dilute to about 20 c.c. Prepare standard solution of ammonium sulphate by diluting 3 c.c. of the standard in a 100 c.c. flask to about 75 c.c. Nesslerise by adding to standard flask 10 c.c. Nessler solution and to the test tube 2.5 c.c., dilute both to appropriate volume (i.e. 100 c.c. and 25 c.c. respectively) and do colorimetric reading.

Calculation. Set standard at 20 m. Divide 20 by reading of unknown and multiply by 15. Result is urea nitrogen in mg. in 100 c.c. blood.

If an autoclave is available and there are many ureas to be done it is much more convenient to hydrolyse 5 c.c. of the filtrate in a hard glass test tube with 1 c.c. normal HCl at 150° for ten minutes. (Cover mouth of test tube with tinfoil.) In the subsequent distillation of the ammonia on account of the acid present 2 c.c. of 10 per cent. sodium carbonate must be used in place of borax.

(If preferred the 0.05 N HCl used for receiving the ammonia distilled over may be titrated with 0.05 N alkali instead of using the Nessler method. The same statement applies to the estimation of non-protein nitrogen if the ammonia is distilled off and received in 0.05 N acid.)

**Determination of Preformed Creatinine.**—Pipette 10 c.c. of blood filtrate into one small flask and into another similar flask 5 c.c. of standard creatinine solution (v. i.) and 15 c.c. water. Add to the blood filtrate 5 c.c. and to the standard creatinine solution 10 c.c. of freshly prepared alkaline picrate solution (25 c.c. saturated solution of pure picric acid mixed with 5 c.c. 10 per cent. sodium hydroxide). Allow both to stand eight to ten minutes then do colour comparison in the ordinary way. This comparison should be completed within fifteen minutes after the addition of the picrate. Also it is advisable to have, if possible, several standard creatinine solutions available in case of meeting with very abnormal blood creatinines. If necessary, however, the unknown may be further diluted by the addition of dilute alkaline picrate solution (1 part solution: 2 parts water).

The standard (blood) creatinine solution. Add from a known solution of pure creatinine (pure creatinine may be prepared by the method of Benedict, *J. Biol. Chem.*, 1914, 18, 183) an amount containing 6 mg. of creatinine and 10 c.c. of normal hydrochloric acid to water contained in a litre flask, mix and dilute to mark. Solution may be kept as stock after addition of a few drops of toluol. 5 c.c. of this solution contain

0.03 mg. creatinine and this amount plus 15 c.c. water represents the standard required for most human bloods, approximately 1-2 mg. per 100 c.c. If blood is known to be rich in creatinine a smaller volume of blood filtrate may be used.

**Calculation.** The reading of the standard in mm. (usually 20) multiplied by 1.5 and divided by the reading of the unknown in mm. gives the amount of creatinine in mg. in 100 c.c. blood. As standard is made up to twice the volume of the unknown 5 c.c. while actually containing 0.03 mg. creatinine corresponds to 0.015 mg. in the blood filtrate.

Folin recommends that in all cases where the colorimeter is used a preliminary comparison be always made between two amounts of the standard solution employed in order to ascertain that the two fields of the colorimeter are equal.

**Determination of Total Creatinine (Creatine and Preformed Creatinine).**—Place 5 c.c. of the blood filtrate in a test tube graduated at 25 c.c. Add 1 c.c. normal hydrochloric acid. Cover mouth of test tube with tinfoil and heat in autoclave at 130° C. for twenty minutes (155° C. for ten minutes suffices). Cool, then add 5 c.c. of the alkaline picrate solution as above, let stand eight to ten minutes and then dilute to 25 c.c. The standard solution is 10 c.c. of the creatinine solution in a 50 c.c. flask, add 2 c.c. normal HCl and 10 c.c. alkaline picrate. Allow to stand for ten minutes, then dilute to 50 c.c.

**Calculation.** Reading of standard in mm. (usually 20) divided by the reading of the unknown and multiplied by 6 gives total creatinine in mg. in 100 c.c. blood. Average value is 5-6 mg. per 100 c.c.

**Determination of Uric Acid.**—To 10 c.c. of blood filtrate in each of two centrifuge tubes add 2 c.c. of a 5 per cent. solution of silver lactate in 5 per cent. lactic acid. Stir with a very fine glass rod. Centrifuge; add a drop of the silver lactate solution to the supernatant fluid, which should remain clear. Decant off as completely as possible the clear fluid and then add to each tube 1 c.c. of a 10 per cent. solution of sodium chloride in 0.1 normal HCl; stir thoroughly. Then add 5-6 c.c. water, again stirring, and the centrifuge till clear. (Addition of salt solution frees uric acid from precipitate.) Transfer the two supernatant fluids to a 25 c.c. volumetric flask by decantation, add 1 c.c. 10 per cent. sodium sulphite solution, 0.5 c.c. 5 per cent. sodium cyanide solution and 3 c.c. 20 per cent. sodium carbonate solution.

Prepare now two standard solutions of uric acid containing different amounts of uric acid. Add 1 c.c. standard uric acid solution<sup>1</sup> to one 50 c.c. volumetric flask and 2 c.c. to another similar flask. To the first flask add in addition 1 c.c. of 10 per cent. sodium sulphite solution and then to each flask 4 c.c. of the acid solution of sodium chloride, 1 c.c. of the sodium cyanide solution, 6 c.c. of the sodium carbonate solution and finally dilute with water to about 45 c.c. Solutions contain 0.1 or 0.2 mg. uric acid.

Now add to the standard solutions and to the unknown 0.5 c.c. of the uric acid phosphotungstic reagent (v. p. 283) to the unknown and 1 c.c.

<sup>1</sup> *Standard Uric Acid Solution.*—Dissolve 1 gm. uric acid in 150 c.c. water by help of 0.5 gm. lithium carbonate in a 500 c.c. flask. Dilute to 500 c.c. and mix. Of this solution take 50 c.c. in a litre flask, add 500 c.c. 20 per cent. sodium sulphite solution, dilute to mark and mix. Store in tightly stoppered bottles.

to each of the standards, mix well, let stand ten minutes, fill to the mark with water, mix again and then do colour comparison.

Calculation. If the weaker standard, for example, be set at 20 mm., then 20 multiplied by 2.5 divided by the reading of the unknown gives mg. uric acid in 100 c.c. blood. Note the blood filtrate taken corresponds to 2 c.c. blood and the standard is diluted to twice the volume of the unknown.

**Determination of Sugar in Blood** (Folin and Wu).—Reagents. 1. To 200 c.c. 10 per cent. NaOH and 200 c.c. water add 35 gms. molybdic acid and 5 gms. sodium tungstate. Boil vigorously for twenty to forty minutes so as to remove nearly all the ammonia present in the molybdic acid. Cool, dilute to about 350 c.c. and add 125 c.c. 85 per cent. phosphoric acid. Dilute to 500 c.c. 2. Alkaline copper solution. Dissolve 40 gms. pure anhydrous sodium carbonate in about 400 c.c. of water and transfer to a litre flask. Add 7.5 gms. of tartaric acid and when this has dissolved add 4.5 gms. crystallised copper sulphate. Mix and make up to a volume of 1 litre. (In the event of a precipitate forming filter this off. Reagent should keep indefinitely.) 3. Standard sugar solutions. Stock solution of 1 per cent. pure dextrose preserved with xylol or toluol. Keeps indefinitely. Prepare from this two solutions, (a) containing 1 mg. dextrose per 10 c.c. (5 c.c. stock diluted to 500 c.c.) and (b) containing 2 mg. dextrose per 10 c.c. (5 c.c. of stock diluted to 250 c.c.). Preserved with xylol or toluol these solutions keep for about one month.

Method. Transfer 2 c.c. of the tungstic acid blood filtrate to a test tube (see later), and to two other similar test tubes (all graduated at 25 c.c.), add 2 c.c. of standard sugar solution containing respectively 0.2 and 0.4 mg. of dextrose. To each tube add 2 c.c. of the alkaline copper solution. Transfer the tubes to a boiling waterbath and heat for six minutes. Then transfer them to a cold waterbath and let them cool, *without shaking*, for two to three minutes. Add to each test tube 2 c.c. of the molybdate phosphate solution. The cuprous oxide dissolves rather slowly if the amount is large, but the whole, up to the amount given by 0.8 mg. of dextrose, dissolves usually within two minutes. When the cuprous oxide is dissolved, dilute the resulting blue solutions to the 25 c.c. mark, insert a rubber stopper, and mix. Mixing must be thoroughly carried out. Make the usual colour comparison in the colorimeter. The depth of the standard (a) (in mm.) multiplied by 100 and divided by the reading of the unknown gives the sugar content in mg. per 100 c.c. blood. Necessary correction must be used for standard (b) which is double the strength of (a).

Folin recommends (*Jour. Biol. Chem.*, 41, 1920, 367) that, in order to prevent reoxidation of the copper, test tubes (200 × 25 mm.) be used in which the bottom part is drawn out to a neck about 4 cm. long and of 8 mm. diameter, leaving a bulb at the bottom of approximately 4 c.c. capacity in order to contain almost exactly the blood filtrate and copper solutions. These tubes can be readily made in the laboratory or may be purchased ready for use. The mixture should just fill the bulb. If the bulb is just too large the volume of fluid may be increased by the addition of diluted alkaline copper solution (1 : 1), but not more than 0.5 c.c. in all may be added (Fig. 202).

If only small amounts of blood are available estimation of dextrose by Maclean's method (*Biochem. J.*, 1919, 13, 135) gives very good results.

**Determination of Cholesterol** (Myers).—1 c.c. of blood is pipetted

into a porcelain crucible containing 4-5 gms. of plaster of Paris, stirred and dried preferably in the oven (about 70° C.) for an hour. It is then emptied into a small paper extraction thimble (4 cm. long), which is next inserted into a short glass tube (2.5 × 7 cm.) with a number of holes in the bottom and sides. This tube is connected with a reflux condenser and the tube and cork (of the condenser) inserted in the neck of a 150 c.c. extraction flask containing 20-25 c.c. chloroform. Extraction is carried on for sixty minutes on an electric hot-plate; the chloroform is then made up to some suitable volume, such as 20 c.c., filtered if necessary, and then a colorimetric estimation is made. 5 c.c. of the chloroform extract is pipetted into a dry test tube and 2 c.c. acetic anhydride (must be anhydrous) and 0.1 c.c. of concentrated sulphuric acid are added. After thorough mixing the solution is placed in the dark (and if necessary in a cool place. Best temperature is 22° C.) for exactly ten minutes to allow the colour to develop and then compared with a 0.005 per cent. aqueous solution of naphthol green B. in a colorimeter, or, if preferred, with a standard prepared simultaneously from pure cholesterol. If the Duboscq instrument is used the cups should be re-mounted in plaster of Paris as the chloroform dissolves the balsam ordinarily employed.



FIG. 202.

Calculation. If the standard naphthol green B. solution be set at 15.5 mm. 0.4 mg. cholesterol treated as above will read 15 mm.

$$\frac{\text{Standard in mm.}}{\text{Unknown in mm.}} \times 0.0004 \times \frac{\text{Dil. of chloroform extract from 1 c.c. blood}}{5} \times 100 = \text{cholesterol}$$

content of blood in per cent. Average is about 0.16 per cent.

**Determination of Fat in Blood** may be carried out by the nephelometric method (using either a nephelometer or a converted Duboscq colorimeter) of Bloor (*Jour. Biol. Chem.*, 1914, 17, 377).

CHAPTER XXIII

MUSCLE AND TISSUES

**Muscle** constitutes one of the commonest foodstuffs, meat being the form in which we take a large proportion of our protein and a considerable amount of our fat.

CHEMICAL COMPOSITION OF MUSCLE (APPROXIMATE)

Water . . . . .	75 per cent.
Protein . . . . .	20-21 "
Extractives . . . . .	0.3-0.4 "
Fat . . . . .	2-3 "
Salts . . . . .	1.0-1.3 "

Proteins are most easily studied in an extract of muscle, which is best prepared by killing a rabbit and, by means of a cannula tied into its aorta, washing with normal saline until blood free. The muscles are

then removed and quickly passed through the mincing machine. The mince is mixed with a 5 per cent. solution of magnesium sulphate, the mixture being placed on ice and left standing all night.

Divide some of the extract provided into two parts, *a* and *b*.

EXPERIMENT I. (*a*) Dilute with four volumes of water, and place in the waterbath at body temperature. A clot forms, leaving *muscle serum*.

(*b*) Add some acetic acid. A precipitate forms. Filter. Neutralise the filtrate with  $\text{Na}_2\text{CO}_3$  solution, and dilute it with water. Place it in the waterbath at  $37^\circ\text{C}$ ., and note that no coagulum forms.

These two experiments show us that the extract contains in solution a substance which is precipitated by acetic acid, and which becomes transformed into an insoluble clot under suitable conditions. This body is protein in nature. Prove this by dissolving the clot in (*a*) in 10 per cent. sodium chloride and applying the protein tests. The soluble body is called *myosinogen*, and the clot *myosin*.

Besides myosinogen the extract contains, however, other proteins.

EXPERIMENT II. Take some of the muscle serum in (*a*), or of the filtrate in (*b*), and half saturate with ammonium sulphate. A precipitate of *globulin* results. Filter off this globulin and test the filtrate for *albumin* by full saturation with  $(\text{NH}_4)_2\text{SO}_4$ , or by coagulation by heat after faintly acidifying.

EXPERIMENT III. Use the saline extract of muscle provided. Heat 5 c.c. carefully in a test tube in the waterbath. Note the temperature of the first signs of coagulation. Filter off coagulum and heat the filtrate, noting the temperature at which the flocculi of a second coagulum appear. It will be found that the first protein (paramyosinogen, also termed myosin) is coagulated at  $47^\circ\text{C}$ .; the second protein (myosinogen, also called myogen) at  $56^\circ\text{C}$ .

Both these bodies serve as the source of the coagulated myosin in muscle. In the clotting associated with rigor mortis it is stated that paramyosinogen is converted directly into myosin; whereas myosinogen is first converted into a soluble form (soluble myosin), which is then turned into insoluble myosin. Soluble myosin can be identified by its low-heat coagulation point ( $40^\circ\text{C}$ .).

The coagulation points of the chief proteins of frog's muscle have already been graphically studied.

EXPERIMENT IV. Show that the *watery* extract of muscle provided contains less protein than the saline extract. It contains albumin, but not globulin. Demonstrate this fact. Coagulate the albumin by heat, and save the filtrate to test for phosphates later.

**Organic Extractives.**—These are organic substances which are soluble in water, but not protein in nature. They may be divided into two classes, (*a*) nitrogenous, (*b*) non-nitrogenous. The chief members of the first group are creatine ( $\text{C}_4\text{H}_9\text{N}_3\text{O}_2$ ) and the purin bodies hypoxanthine ( $\text{C}_5\text{H}_4\text{N}_4\text{O}$ ) and xanthine ( $\text{C}_5\text{H}_4\text{N}_4\text{O}_2$ ). The most important non-nitrogenous extractive is sarcolactic acid ( $\text{C}_3\text{H}_6\text{O}_3$ ).

**Creatine** is the most abundant extractive in muscle, amounting to .4–.45 per cent. in rabbits, .3 per cent. in oxen, .26 per cent. in frogs, and .2 per cent. in hedgehogs. Chemically it is closely related to urea, and can be changed into this body and a substance called sarcosine by boiling with baryta water.

Another interesting relationship of creatine is to the substance called *creatinine*. If creatine be boiled with dilute mineral acids it loses a



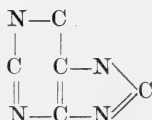
molecule of water and becomes changed into creatinine. This reaction is the best means of recognising creatine.

**EXPERIMENT V.** Take about 10 gms. of fresh muscle, grind with alcohol, filter, evaporate at about 50° C. Dissolve in water and divide into two equal portions, *a* and *b*. To *a* add 15 c.c. of saturated picric acid solution and 5 c.c. of 10 per cent. caustic soda. Allow to stand 5 minutes and dilute to 500 c.c. Note that there is no change in the colour of the solution, therefore creatinine is absent. To *b* add half its volume of N. HCl and heat in a flask fitted with a cork and glass tube to act as air condenser on water-bath for five hours. Neutralise with caustic soda, add picric acid solution and caustic soda, and dilute as before. Note red colour, due to picramic acid. *Creatinine is now present.* (For other tests for creatinine see chapter on Urine.)

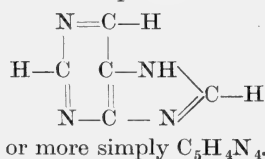
**Diacetyl Test for creatine.** To 2 c.c. of a watery protein free extract of muscle in a test tube add 2 c.c. of saturated Na<sub>2</sub>CO<sub>3</sub> solution, 6 drops of freshly prepared diacetyl solution and 2 c.c. of water. Immerse in boiling water for 1 minute. A red colour develops.

Diacetyl solution is prepared as follows :—1 grm. Dimethyl-glyoxime, 100 c.c. water and 20 c.c. conc. H<sub>2</sub>SO<sub>4</sub> are placed in a flask and slow distillation is carried out until 50 c.c. collected. The solution must be used fresh.

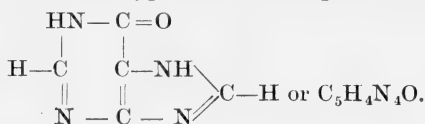
**Hypoxanthine and Xanthine.**—These are members of the group of bodies known as the purine bodies. They are thus termed because the so-called purine ring



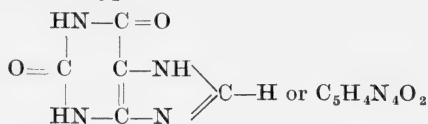
is the basis of their constitution, purine itself, a synthetic body being



Hypoxanthine is 6-monoxypurine, and is represented by the formula



Xanthine is 2, 6-dioxypurine :



Lastly, 2, 6, 8-trioxypurine, which occurs in muscle in traces only, is *uric acid*, C<sub>5</sub>H<sub>4</sub>N<sub>4</sub>O<sub>3</sub>. (See Chapter XV, p. 259.)

Hypoxanthine and xanthine result in part from the breakdown of the nuclein present in the muscle, but their amount is normally so large compared to the amount of nuclein present that this cannot be their sole source; the other source of supply is at present unknown.

**Lactic Acid** ( $C_3H_6O_3$ ).—This variety of lactic acid differs from that obtained by the fermentation of lactose, which does not rotate the plane of polarised light. The lactic acid of muscle, often termed *sarcoplactic acid*, rotates the plane of polarised light to the right. The amount of lactic acid increases **markedly** during the death of a muscle, and also during muscular activity. These points can be shown by the following experiments:

(a) To some of Uffelmann's reagent (a mixture of ferric chloride and carbolic acid) add some of the muscle extract provided. This probably contains lactic acid from the dying muscle; if it does the violet colour of Uffelmann's reagent will be turned to yellow by the lactic acid present.

(b) *Hopkins' Test for Lactic Acid*. Take about 5 c.c. of strong sulphuric acid in a dry test tube, add 1 or 2 drops of a solution of muscle extract, 2 or 3 drops of saturated solution of copper sulphate. Warm in boiling water for about two minutes; cool and add a few drops of alcoholic thiophene solution (20 minims in 100 c.c. alcohol), and warm gently. With lactic acid a cherry red colour develops.

(c) Take a pithed frog which has been kept on ice for half an hour. Quickly cut off the muscles of one hind limb; cut off the other limb at the pelvic girdle, and stimulate electrically until irritability is nearly lost. Cut off the muscles. Treat both sets of muscles as follows: Grind with cold absolute alcohol and sand, filter, evaporate the alcohol, dissolve in water, heat with a little animal charcoal, filter, evaporate, and apply the thiophene test. It will be found that the muscles of the tetanised limb give a positive reaction; those of the non-tetanised do not.

Another important nitrogen-free extractive is **glycogen** ( $C_6H_{10}O_5$ )*n*. The relative amount of this is small (0.5 to 1 per cent.), but it varies in different animals, and is much diminished after muscular activity.

Other extractives are: **Urea**, **carnosine**, **dextrose** (trace), **inositol** (hexahydroxybenzene), and **lecithin**.

**Inorganic Salts**.—These consist of salts of the alkalis and alkaline earths. The chief acid radicle present is *phosphoric acid*, and this exists in several states—(a) Inorganic phosphates, (b) phosphorus of lecithin, (c) phosphorus of nuclein, (d) phosphorus of other organic compounds.

EXPERIMENT VI. The watery extract of muscle has been freed of proteins by boiling it. Add to the clear filtrate an ammoniacal solution of magnesium citrate. A white precipitate of phosphates results. Show that this precipitate consists of phosphates by dissolving it in nitric acid and testing with ammonium molybdate.

**Preparation of Extractives of Muscle**.—500 gms. of meat, from which as much fat and tendon as possible have been removed, are finely minced; the mince is thoroughly mixed with 500 c.c. of water and heated for half an hour on a waterbath at 50° C. The extract is strained through muslin and the residue extracted several times in a similar manner, the extracts being mixed together. The protein in the extract is then coagulated by boiling, and, after cooling, the coagulum removed by filtration.

A similar extract may be prepared by dissolving some commercial meat extract in water.<sup>1</sup>

To remove the phosphates and the last traces of proteins from this extract a saturated solution of subacetate of lead is added to it until no more precipitate is produced. (Care should be taken that an excess of the subacetate solution is not added. This may be ascertained by filtering samples of the extract and seeing if these yield further precipitates with the subacetate solution.) The precipitate thus obtained is removed by filtration.

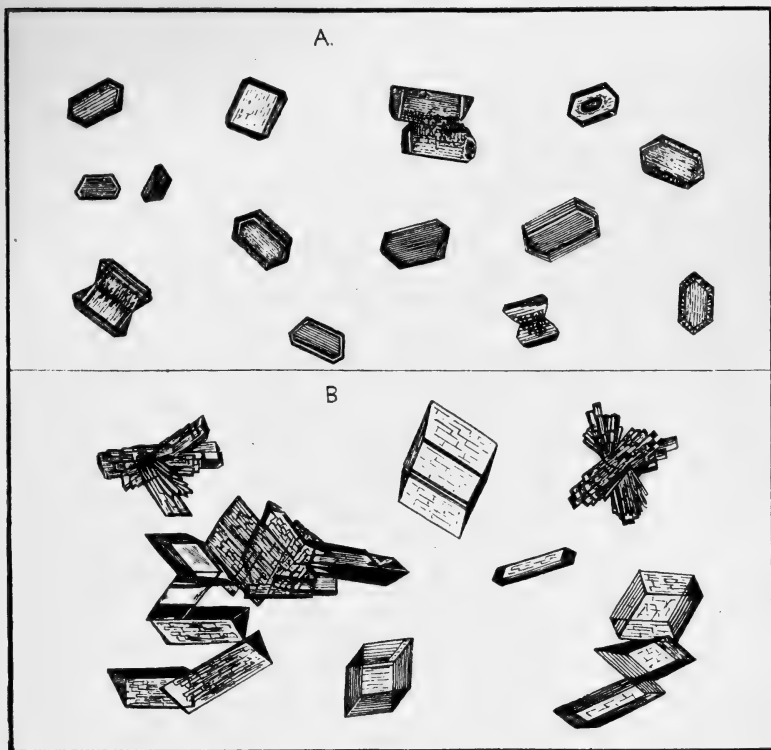


FIG. 203.—Crystals obtained from meat extract, mostly creatine, a few sodium chloride.

The excess of lead is precipitated from the filtrate by passing a current of sulphuretted hydrogen through it. The precipitate of lead sulphide is removed by filtration. The filtrate is then evaporated to small bulk

<sup>1</sup> The following amounts are suitable for this preparation: 10 gms. bovril or lemco are dissolved in 200 c.c. water, and to this is slowly added 60 c.c. of a saturated solution of subacetate of lead. After the precipitate has settled down a sample of the supernatant fluid is removed by a pipette to a test tube and tested with the subacetate solution to be certain that no more precipitate is produced.

(any sulphur which may separate out being removed by filtration) and allowed to stand on ice for two or three days, when a large number of oblique rhombic crystals of creatine will have separated out. (See Fig. 203.) These are collected on a filter (for which purpose a suction pump will be found necessary) and are thoroughly washed with alcohol until no more pigment is removed. The filtrate is preserved for the isolation of the other extractives.

**Xanthine and Hypoxanthine.**—The creatine-free filtrate is made strongly alkaline with ammonia, and silver nitrate solution added until precipitation is complete. The purine bodies are thus precipitated. The precipitate is collected on a filter paper and thoroughly washed with dilute ammonia. The hypoxanthine and xanthine are separated by the following method: the precipitate is removed from the filter paper and dissolved in boiling nitric acid (sp. gr. 1.1), a few crystals of urea being added to the solution so as to destroy any nitrous acid which may be present, and which would decompose the purine bodies. When all the precipitate has dissolved the solution is quickly filtered hot, and the filtrate is allowed to stand overnight, when it will be found that a precipitate consisting of fine needle-shaped crystals (Fig. 204) has separated out. This consists of hypoxanthine silver nitrate combined with nitric acid; to remove the nitric acid wash it with distilled water, transfer it from the filter to a small beaker and boil it with ammonia until the crystals break up and become amorphous, and then, to remove the silver, pass in  $H_2S$ , filter off the silver sulphide, and evaporate the filtrate slowly to dryness, when a white chalk-like mass of *hypoxanthine* will be obtained. In order to obtain the xanthine silver salt the filtrate from hypoxanthine should be treated with ammonia, when a few yellow flakes of the salt will be obtained. To separate the *xanthine* this precipitate is treated in exactly the same way as for hypoxanthine.

**Test for Hypoxanthine.**—Place some hypoxanthine in a small evaporating dish with a few drops of pure concentrated nitric acid and evaporate slowly to dryness: a brilliant yellow residue is obtained. Cool, and then add a drop of sodium hydrate solution, when the residue will change to orange. If the residue be dissolved in water and the solution again evaporated to dryness the orange colour persists, thus differing from the murexide stain which, when similarly treated, loses its colour (see p. 259).

**Test for Xanthine.**—Repeat the same test as for hypoxanthine and note that the sodium hydrate produces in this case a deep red colour, which persists on dissolving in water and evaporating.

**Sarcolactic Acid.**—The ammoniacal filtrate, from which the alloxuric bodies have been separated, is treated with sulphuretted hydrogen gas so as to remove the silver which it contains: the silver sulphide is filtered off, and the filtrate evaporated till all the ammonia has been expelled. It is then made strongly acid with phosphoric acid, and the lactic acid, which is hereby liberated, is dissolved out by shaking it in a separating funnel with ether.

After extracting three or four times, the ethereal extracts are combined and the ether evaporated away by placing on a waterbath heated to about  $60^{\circ}C.$ , the flame underneath which has been extinguished. An acid syrup remains behind; this is impure lactic acid. In order to purify it, dilute three times with water, bring the

resulting solution to the boil, and then carefully add powdered zinc carbonate until the reaction is neutral. Filter. Evaporate the filtrate to small bulk, add an equal bulk of spirit and allow to stand, when *zinc sarcocollate* will crystallise out (Fig. 205). The zinc salt is filtered off, washed several times with spirit, dissolved in water,<sup>1</sup> and the zinc separated by passing a stream of sulphuretted hydrogen through the solution. The zinc-free filtrate is then freed of water by evaporation, when the *lactic acid* is obtained as a syrup (see also pp. 294, 322).

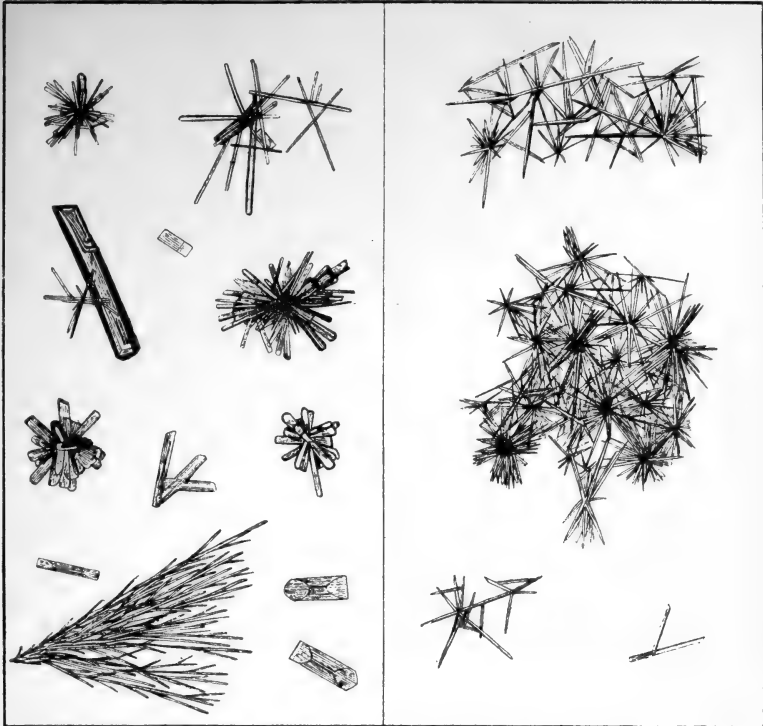


FIG. 204.—  
Hypoxanthine silver nitrate.  $\times 300$ .

FIG. 205.—  
Zinc sarcocollate.  $\times 300$ .

**Preparation of Nucleo-Protein.**—Boil 100–200 gms. finely minced pancreas with about a litre of water for ten to fifteen minutes and then filter. To the filtrate, whilst still warm, add acetic acid drop by drop until a finely-divided precipitate begins to settle out. Filter this precipitate off, wash with water, then very thoroughly with alcohol and finally with ether. It is best to allow the precipitate to stand for some hours in ether, then dry. The dry substance is nucleo-protein.

**Detection of Pentose in the Nucleo-Protein.**—To a small quantity

<sup>1</sup> The watery solution should be evaporated until the crystals of zinc sarcocollate begin to appear, this being ascertained by examining a drop under the microscope.

of the substance in a test tube add several c.c. HCl and a knife point of orcin, then boil. A reddish blue colour develops (a blue pigment is formed). Extract with amyl alcohol—the colour changes from reddish to greenish. Examine with a spectroscope. Bands are found between C and D. The addition of a trace of ferric chloride to the hydrochloric acid renders the test more delicate.

Presence of phosphorus may be shown by means of the general test for P. The amount may be estimated by the Neumann wet ash method (see p. 289).

Purin bodies present may be obtained by hydrolysis with a mineral acid and subsequent treatment as in isolation from muscle extracts.

**Detection of Pentoses in Gums.**—Hydrolyse gum arabic by heating a solution of it in a waterbath for twenty minutes with 5 per cent. HCl. Arabinose is formed. After neutralising, apply reduction and yeast fermentation tests to portions of the solution. To another portion apply the following characteristic test for pentoses (Tollens). Add phloroglucin ( $C_6H_3(OH)_3$ ) in small quantities at a time till no more dissolves to a solution of about 5 c.c. of equal parts of concentrated HCl and water. Then add a few drops of the arabinose solution and warm until a red colour develops. Examine with the direct vision spectroscope when an absorption band will be seen between D and E lines. By further heating, a precipitate forms which becomes dissolved in amyl alcohol when this is shaken with the solution. The amyl alcoholic solution shows the above spectrum very clearly. Tollens' test can be applied to urine. Repeat this test, using dextrose solution.

**The Quantitative Estimation of Glycogen in Animal Tissues.**—The best method is that of Pflüger. This method depends on two facts: firstly, that glycogen is not affected by heating it on a waterbath with 30 per cent. potassium hydroxide solution, whereas protein under such conditions is destroyed; and secondly, that by the addition of an equal volume of water to the above solution (which will bring the percentage of potassium hydroxide to 15) and the subsequent addition of two volumes of alcohol (96 per cent.) all the glycogen is precipitated, whereas practically all of the degradation products of protein remain in solution. The method is as follows: <sup>1</sup>

The liver is cut into small pieces and mixed in an Erlenmeyer flask (Bohemian glass) with 100 c.c. 60 per cent. KOH.<sup>2</sup>

The flask is closed with a cork, having a wide glass tube about five feet long passing through it to serve as a reflux condenser, and it is then immersed in a boiling waterbath and left there for three hours, with occasional shaking. (Less time than this suffices to completely destroy the protein of liver.) On removal from the waterbath, the contents of the flask are allowed to cool, and are then thoroughly shaken, with 200 c.c. water (thus bringing the percentage of KOH to 15). 800 c.c.

<sup>1</sup> The following description is for 100 gms. liver, but much less than this amount is sufficient for most purposes. Thus, in the case of a dog fed on the previous day with bread and meat, 20 gms. liver is a suitable amount, and in the case of a rabbit fed with carrots or other carbohydrate-rich food, 10 gms. is sufficient. In the case of muscle, it is best to take 100 gms., as the percentage of glycogen in this tissue is practically never more than one.

<sup>2</sup> Pflüger specifies "Merck A" KOH, but for most purposes "KOH pure by alcohol" is of sufficient purity. The strength is best adjusted by the use of a hydrometer (alkalimeter), the specific gravity of such a solution being 1.438 at 15° C. or 44 on the Beaumé scale.

of ordinary (96 per cent.) alcohol are then added to the solution, the mixture shaken and allowed to stand for several hours (preferably overnight).

The more or less white precipitate of glycogen will by this time have settled down, so that the supernatant reddish fluid can with care be poured off into a beaker, after which it is filtered through a filter paper of suitable size, so as to collect on the filter any particles of glycogen which the decanted fluid may contain. The precipitate of glycogen is now thoroughly shaken with about ten times its volume of 66 per cent. alcohol (about 700 c.c. alcohol and 300 c.c. water) containing 1 c.c. per litre of a saturated solution of NaCl. This washing fluid removes many of the impurities which adhere to the glycogen.

After settling, the wash fluid is decanted into the same beaker as was employed for receiving the original supernatant fluid, and filtered through the same filter. This process is repeated at least once again, after which the precipitate is shaken with ordinary alcohol (about ten times its volume), and the suspension thrown on to the same filter paper as used above.

When the alcohol has all drained off, the precipitate is washed on the filter paper with ether. All the washed glycogen has thus been collected on the filter paper and must now be dissolved, for which purpose the filter is filled up with boiling water, and the solution of glycogen allowed to filter through into a clean Erlenmeyer flask. When the first added water has completely drained through the filter, the filter is filled up with boiling water a second and a third time. It is essential to allow the filter to drain completely before adding more water. To be certain that all the glycogen has been dissolved, some of the final filtrate should be tested with alcohol for glycogen.

The resulting opalescent solution can now be employed either for the preparation of pure glycogen or for its quantitative estimation. For the former purpose the glycogen is precipitated by alcohol; for the latter purpose the glycogen solution is made up to a litre in volume, and of this 200 c.c. are taken, mixed with 10 c.c. HCl (conc.) (i.e. 5 c.c. HCl to 100 c.c. of glycogen solution), and heated in a flask on the waterbath for three hours.<sup>1</sup> Complete hydrolysis of the glycogen is certain within this time, although the resulting solution often contains a flocculent precipitate which is probably protein in origin. The solution, after cooling, is neutralised with 20 per cent. KOH and filtered into a 250 c.c. measuring flask through a small filter (10 cm.) paper.

The flask used for inversion is rinsed three times with distilled water, the washings being each time poured on to the filter and added to the contents of the measuring flask. In this way the volume of the dextrose solution is brought exactly to 250 c.c.

Where only 10 or 20 gms. of liver were originally employed, the above measurements must of course be altered, it being usually best to take all of the glycogen solution for inversion and bring it to a definite volume after neutralising.

The estimation of the sugar may be carried out either by a volumetric or a gravimetric method.

**Estimation of Cholesterol in Tissues.**—Based on the discovery of

<sup>1</sup> If the glycogen be reprecipitated and redissolved in a known volume of water the resulting solution can be examined in the polarimeter and its glycogen content calculated according to the formula on p. 214,  $(\alpha)_D = + 196.63$ .

Windaus that cholesterol, but not cholesterol esters, readily combines with digitonin to form a very insoluble compound, methods have been introduced for the quantitative estimation of cholesterol by gravimetric methods. One of the simplest is that used by Gardner.

Grind up the tissue with sand and a sufficient amount of plaster of Paris to make it set. When the mass is dry it is finely powdered and thoroughly extracted with ether in a Soxhlet apparatus. (Extraction requires several days at least.) The ethereal extract is then evaporated to dryness, the residue weighed and taken up in 95 per cent. alcohol. To this solution is added an excess of digitonin in 95 per cent. alcohol and the mixture after standing for some time is evaporated to dryness in a vacuum desiccator. The precipitate is next washed, by decantation with ether, into a previously weighed filter paper or Gooch crucible until the washings give no residue on evaporation. The excess of digitonin is next removed by washing with warm water until the washings give no precipitate on evaporation. The precipitate is then dried in an air oven at 110° C. and weighed, both drying and weighing being carried out in stoppered glass bottles as the compound is somewhat hygroscopic.

If it be desired also to determine the amount of cholesterol present in ester form the ethereal washings from the above estimation are saponified with excess of sodium ethylate, the unsaponifiable matter dissolved in alcohol, precipitated with digitonin and treated as above. Or preferably, if material permit, it is better to do two estimations, (a) total free and (b) total free and combined cholesterol present, then (b) minus (a) will give combined cholesterol.

Cholesterol has also been estimated by colorimetric methods (see Blood, p. 318).

**Preparation of Glucosamine from Chitin.**—Soak lobster or crab shells in dilute (2 per cent.) hydrochloric acid until all calcium carbonate is removed and the material is soft and pliable. Wash well with water, then cut into small pieces, place them in a round-bottomed flask, add concentrated hydrochloric acid and boil gently under a reflux condenser for four hours. The resultant solution is then evaporated on the waterbath in an evaporating basin until crystallisation commences; allow to cool. Filter off the crystal mass, wash carefully with a small quantity of cold water and dilute alcohol. The crystals are again dissolved in water and the solution again evaporated until commencing crystallisation. Filter off the crystals: to free them from any inorganic material dissolve the mass in 80 per cent. alcohol, filter, and again evaporate to crystallisation. The crystal mass of glucosamine hydrochloride which is formed should be almost pure. Glucosamine forms an osazone identical with glucosazone. Its solutions reduce Fehling's solution.

#### CHAPTER XXIV

#### FOODSTUFFS AND METABOLISM

**Milk.**—Milk contains proteins, fats, carbohydrates, salts and water. The fat is suspended in the form of a fine emulsion. The proportion of these bodies varies in the milk of different animals. Naturally that provided by the animal is the best for its own species.



In everyday life the two milks of the greatest importance are cow's milk and human milk. These two milks vary in composition :

Milk.	Water.	Protein.	Fat.	Carbohydrate.	Salts.
Cow.	87.8	3.4	3.7	4.7	0.7
Human	87.6	2.0	3.2	6.4	0.3

The milks also differ in that (1) the proportion between the amount of the kinds of protein in cow's and human milk is different.

Cow's milk, 2.7 per cent. caseinogen, .50 per cent. lact-albumin.  
 Human ,, .80 ,, ,, 1.21 ,, ,,

It will be seen that human milk contains far more lact-albumin than does cow's milk, so that even when cow's milk is diluted there is the discrepancy between the relative amount of the proteins to be taken into account.

(2) The caseinogens of the two milks are not of the same composition either in percentage or actual composition.

(3) The percentage of the salts present differs in the two milks—

ASH CONTENT.

	Human.	Cow.
K <sub>2</sub> O . . . . .	0.077	0.17
Na <sub>2</sub> O . . . . .	0.021	0.05
CaO . . . . .	0.032	0.18
MgO . . . . .	0.006	0.02
Fe <sub>2</sub> O <sub>3</sub> . . . . .	0.0005	0.001
P <sub>2</sub> O <sub>5</sub> . . . . .	0.047	0.26
Cl . . . . .	0.043	0.10

In order to study the chemistry of milk, we usually employ cow's milk, because it is easily obtainable.

**Cow's Milk.**—This is an opalescent solution, possessing a characteristic taste, and of amphoteric reaction.

EXPERIMENT I. Place a drop of fresh milk on a piece of glazed red litmus paper, and wash it off with distilled water ; a blue stain is left : if the drop be placed on blue litmus, a red stain is left. This peculiar reaction is due to the fact that milk contains a mixture of acid and alkaline salts. By ascertaining how much decinormal acid or alkali are required to produce neutralisation with the aid of different indicators the amount of each of these kinds of salt can be determined.

The *specific gravity* of fresh milk varies between 1.028 and 1.0345. The more fat (i.e. cream) the milk contains the lower is the specific gravity.

EXPERIMENT II. Estimate by a hydrometer the specific gravity (a) in skimmed milk and (b) in fresh milk. In the former it is about 1.0345, in the latter 1.028. By adding water to (a) the specific gravity obviously falls, and by removing the cream from (b) it rises.

Fresh milk does not coagulate on boiling, but a skin forms on its surface. A similar skin is produced when any emulsion containing protein is boiled, and in the case of milk it is composed in part of

caseinogen entangling some fat globules.<sup>1</sup> Its formation is due to drying of the protein at the surface of the milk.

### The Chemical Constituents of Milk

**I. Proteins.**—The main protein of milk is a phospho-protein called *Caseinogen*. This can be precipitated by adding to the diluted milk a weak acid, or by saturating it with a neutral salt. (See Phospho-proteins, p. 198.)

**EXPERIMENT III.** Place about 5 c.c. of milk in a test tube, and dilute with an equal bulk of water. To this diluted milk add, drop by drop, a weak solution of acetic acid; a precipitate of caseinogen, entangling fat, falls down. Filter off this precipitate and wash it with water. Now add to it a weak solution of  $\text{Na}_2\text{CO}_3$ ; the precipitate dissolves, and an opalescent solution of caseinogen, still, however, containing some fat, passes through the filter. By repeated reprecipitation and filtration comparatively pure caseinogen can be obtained, from which the last traces of fat can be removed by treating with ether.

The chief property of caseinogen is its power to clot when treated with *rennin* (a ferment contained in gastric juice) in the presence of soluble calcium salts. (See Digestion, p. 232.)

The fluid left after the clotting of the caseinogen is known as whey—in this case rennet whey. If the caseinogen be got rid of by acid, it is known as “acid whey”; if by “salting out,” as “salt whey”; if by alcohol, “alcoholic whey,” and so on. These wheys are different in composition; for instance, rennet whey and acid whey contain lact-albumin, salt whey and alcoholic whey do not.

**EXPERIMENT IV.** Apply the xanthoproteic reaction to some acid whey: a positive result is obtained. Apply also the other protein colour tests. Acidify some of the whey with acetic acid and boil; the protein is coagulated. The proteins are called lact-albumin and lact-globulin.

**II. The Carbohydrate—Lactose.**—**EXPERIMENT V.** Boil some rennet whey which has been weakly acidified with acetic acid. Filter off the coagulated proteins. To the filtrate apply Trommer's or Fehling's test; reduction is effected. Barfoed's reagent is not reduced.

Lactose does not, like dextrose, readily ferment with yeast, but it is capable of undergoing a special fermentation, which changes it into lactic acid. This is called the lactic acid fermentation. It depends on the presence of a microbe, the *bacillus acidi lactici*.

The presence of these free acids in the milk leads to the *precipitation* of caseinogen, and this explains the production of the *curd* in sour milk. It is quite a different thing from the *curd* which is produced by rennin. Thus, it can be dissolved by means of a weak alkali, and if rennin be added to the resulting solution true clotting will follow.

Milk, however, will undergo alcoholic fermentation by a special fungus, known as the kephir fungus. From cow's milk the drink *kephir* is formed, from mare's milk the drink *koumiss*. They contain from 1–3 per cent. of alcohol, and when clotted give a fine clot.

**EXPERIMENT VI.** Take some sour whey. Add a few drops of it

<sup>1</sup> An emulsion of cod-liver oil in diluted blood-serum is given out; warm it to about 50° C., and a skin will form on the surface. Be careful not to heat above 50° C., as then coagulation of the proteins will be produced.

to Uffelmann's reagent,<sup>1</sup> when the dark purple colour of the latter will be changed to yellow. Test for lactic acid (see p. 230).

**III. The Fats of Milk.**—Examine a thin film of milk under the microscope, and note that the fat consists of small spherical bodies, which are transparent and do not adhere to one another.

The fat can be removed by shaking the milk with ether after the addition to it of a few drops of weak NaOH solution.

**EXPERIMENT VII.** To about 5 c.c. of milk in a test tube add two drops of caustic soda (20 per cent.), and then about 5 c.c. of ether. Cover the top of the tube with the thumb and shake the mixture, occasionally lifting the thumb slightly to allow the vapour of ether to escape. The ether will dissolve the fat, and the milk will become much less opaque. By adding alkali, a certain amount of the caseinogen is changed in its physical condition, so that the caseinogen films, which lie between and thereby hold apart the fat globules, are diminished, and consequently the fat globules are dissolved by ether. So long as they are surrounded by caseinogen molecules they are not acted on by ether. Not only alkalis, but also acids can effect this change.

**Colostrum.**—The milk which first appears during lactation is yellower in colour and of higher specific gravity than that secreted later. On boiling, it yields a distinct coagulum of albumin and globulin, and if examined under the microscope it will be found to contain numerous cells—*colostrum corpuscles*—in the protoplasm of which fat globules are present. These cells are, in reality, secretory cells of the mammary glands which have been extruded in the first portions of milk.

**IV. The Salts.** **EXPERIMENT VIII. THE DETECTION OF PHOSPHATES AND CHLORIDES.**—Add to 5 c.c. of protein-free whey half its bulk of nitric acid and about twice its bulk of a solution of molybdate of ammonia in nitric acid. Warm gently on the waterbath, and a yellow precipitate of *phosphate* forms. In rennet or acid whey the phosphates may be precipitated by ammoniated magnesium citrate. Filter. Dissolve precipitate in nitric acid and heat as before with ammonium molybdate. Show the presence of chlorides by means of silver nitrate test—a white precipitate insoluble in nitric acid, soluble in ammonia.

**EXPERIMENT IX. THE DETECTION OF CALCIUM SALTS.**—To some whey, freed from protein by boiling, add a few drops of a solution of potassium oxalate—a white haze of calcium oxalate results.

**The Quantitative Determination of the various Bodies in Milk.**—The methods here described can be employed for other fluids besides milk.

(1) **The Percentage of Water.**—A weighed quantity of milk is mixed with a weighed quantity of fine quartz sand, which has been previously heated to redness and then cooled in a desiccator. The weight of the mixture is accurately determined, and it is then placed in a hot air bath heated to 100° C. until all the water has been driven off and the weight is constant. The amount of weight lost corresponds to the amount of water which the sample of milk contains.

(2) **The Percentage of Protein.**—Three gms. of milk are diluted with four times its volume of distilled water, a few c.c. of a solution of sodium chloride are added, and then a solution of tannic acid until all the protein

<sup>1</sup> This reagent is made by adding a trace of ferric chloride to a 1 per cent. solution of carbolic acid.

has been precipitated. The precipitate is filtered off through an ash-free filter paper, and thoroughly washed with distilled water. The filter paper with the precipitate is removed to a Kjeldahl's combustion flask, and the nitrogen estimated as described on p. 279. The result multiplied by 6.37 gives the total amount of protein contained in the sample of milk.

A simpler and yet fairly accurate method of estimating protein is by titration after the addition of formaldehyde. To 10 c.c. of milk add  $\frac{N}{10}$  NaOH till neutral to phenolphthalein; 2 c.c. of neutral 40 per cent. formalin are added and the mixture titrated with  $\frac{N}{10}$  NaOH to the same neutral tint as before. The number of c.c. of tenth normal soda used, multiplied by 0.17, gives a close approximation to the amount of total proteins. If greater accuracy is required it is advisable to use tenth normal strontium hydroxide instead of sodium.

(3) **The Percentage of Fat.**—The following method (Adam's) will be found very simple and sufficiently accurate for most purposes:

Measure 5 c.c. milk and drop it on to a strip of Adam's fat-free porous paper;<sup>1</sup> allow this to dry in the air bath at 60° C., then roll it up and place it in the extractor of Soxhlet's apparatus (see p. 299). The weight of the distilling flask is ascertained before beginning the extraction, and then again after the extraction has been allowed to proceed for about one hour and the ether has been distilled off; the increase of weight gives the amount of fat in 5 c.c. of milk. Sufficient ether should be used to fill the Soxhlet one and a half times, and it should be made to siphon over at least twelve times.

A more rapid and yet good method of estimating fat in milk is a slight modification of the Werner-Schmidt method. Place 10 c.c. of well-mixed milk in a Stoke's tube, which is graduated to 50 c.c. Add 10 c.c. strong hydrochloric acid (to liberate all fat), close the tube with a cork and heat it in a boiling waterbath for about ten minutes, shaking from time to time. The colour of the mixture should be a dark chocolate brown. Cool now under the tap and when quite cold fill up to the 50 c.c. mark with alcohol-free ether (washing ether with water frees it from alcohol). Insert cork and shake mixture vigorously for one minute. When the ether has again risen to the surface a small layer of undissolved matter will be seen between the ethereal and the acid fractions. Read off the volume of the ether from the middle of this layer to its upper surface. Take of the ethereal solution two 10 c.c. samples and place in weighed porcelain dishes. Evaporate to dryness, then heat to constant weight in a hot-water oven. Percentage of fat is calculated from the mean of the two weighings. Weight of fat multiplied by volume of ether solution and divided by 10 gives fat content in amount of milk originally used.

(4) **The Percentage of Sugar.**—Folin's method of estimating sugar has been found to give good results (see p. 275).

In determining the amount of lactose in milk it is convenient to dilute with water as follows: Cow's milk 1 : 4, human milk 1 : 5 or, if only a small amount is available, 1 : 6. 5 c.c. of Folin's reagent = 40.4 mg. lactose.

(5) **The Percentage of Ash.**—A weighed quantity of milk is evapor-

<sup>1</sup> The paper can be obtained from any of the dealers.

ated to dryness on a waterbath in a weighed crucible. The crucible is carefully heated over a free flame until a perfectly dry and black ash has been obtained. The flame is now strengthened and the ash is heated until it becomes white. The crucible is then allowed to cool in a desiccator, after which it is weighed.

**Flour.**—

I. Determine moisture and ash by ordinary methods.

II. Determine total nitrogen by Kjeldahl method. Use about 1 gm. of flour.  $T.N. \times 5.7$  gives gluten content.

III. Determine nature of proteins present.

Make about 30 gms. of flour into a stiff dough with 12–15 c.c. water and allow the mass to stand for an hour. It is then carefully kneaded, in a stream of water, over a sheet of fine muslin, which allows the starch to pass through, but retains any particles of gluten, or the mass may be wrapped in linen and then kneaded. The ball of gluten thus obtained is tough and elastic and can be pulled out into threads. After washing the ball of gluten is left for an hour under water, and then the excess of moisture removed by squeezing. Gluten consists of two proteins: gliadin, which is soluble in dilute alcohol, and glutenin which is soluble in very dilute alkali. If the mass of crude gluten obtained as above be extracted with 70 per cent. (by volume) alcohol gliadin goes into solution and can be precipitated by the addition of sodium chloride. The amount present in gluten may be estimated by carrying on the alcohol extraction for two hours and determining the amount of nitrogen in the filtrate. The nitrogen value multiplied by 5.7 gives the gliadin content.

IV. Determination of carbohydrate.

3 gms. of the flour are extracted with 50 c.c. of cold water for an hour with frequent stirring. The mixture is filtered (if there is any difficulty the addition of 2 c.c. of alumina cream helps) and the residue washed with water until filtrate equals 250 c.c. The soluble carbohydrate can be estimated in the filtrate both before and after hydrolysis. The insoluble residue is transferred to a flask, fitted with a reflux condenser, 200 c.c. of water and 20 c.c. strong HCl (sp. gr. 1.125) are added. Heating is continued for two and a half hours and then the contents are cooled, nearly neutralised with strong NaOH, made up to 250 c.c., filtered, and the dextrose determined in an aliquot portion of the filtrate. The gravimetric Fehling method (see p. 304) serves excellently. Dextrose value multiplied by 0.9 gives weight of starch.

**Egg.**—The nature of the proteins present and their coagulation temperature can be determined in “white of egg.”

The presence of lipoids can be determined in egg-yolk which contains fat, lecithin, cholesterol and a phospho-protein vitellin.

Extract the yolk with ether by repeated shaking in a flask. Cautiously evaporate, by placing the porcelain basin containing the ethereal extract on a previously heated waterbath (*use no flame*) or preferably on an electric hot-plate, to small volume. Add acetone until a distinct precipitate is formed—crude lecithin. Filter off the precipitate. Keep the filtrate which contains cholesterol (and some lecithin).

Precipitate. Dissolve the precipitate by means of alcohol and drop slowly, with stirring, the alcoholic solution into water. A white precipitate results—“lecithin.” Saponify some of this emulsion by heating with caustic alkali. (Note odour of trimethylamine.) Addition

of strong hydrochloric acid causes separation out of the fatty acids. The presence of phosphorus in this emulsion can also be determined.

Nervous tissue (brain) which has been previously dried serves as an excellent source for lipid material. A rough separation may be carried out by first extracting the material with cold acetone which takes out the cholesterol, then with cold ether which removes mainly phosphatides (do P. test) and finally with hot alcohol or chloroform which extracts the cerebrosides. Test last extract for presence of a reducing sugar—galactose (see p. 215).

**Pulses.**—Determine presence of a native protein.

Pea flour, 10–20 gms., is stirred up with twice its weight of 10 per cent. NaCl solution and allowed to stand for at least one hour. The starch is then filtered off and the filtrate saturated with ammonium sulphate. The precipitate which results is a mixture of two globulins, legumin and vicilin. These may be separated from their solution in very dilute ammonium sulphate, as legumin is precipitated on  $\frac{1}{10}$  saturation with ammonium sulphate (i.e. addition of 150 c.c. saturated ammonium sulphate solution to 100 c.c. of the globulin solution). The ammonium sulphate can be got rid of from the precipitate by dialysis. Prove globulin nature of legumin.

The table on following page gives a summary of the composition of some of the chief foodstuffs. The data have been taken from the recent analyses of R. H. A. Plimmer (*Analyses and Energy Value of Foods*, H.M. Stationery Office, 1921).

**The Methods for the Estimation of General Metabolism.**—In carrying out the examination of the changes which take place in the organism, whether the subject be starving or fed, there are three lines of attack. Obviously if all three methods can be used at once the results obtained are most comprehensive. The three lines of attack are the investigation of the excreta (urine and fæces), the respiratory exchange by way of the lungs, and the heat loss. The direct determination of heat loss may be ruled out at once as a practicable method as it involves the construction of a most costly calorimeter. The other two channels of excretion fortunately enable us to determine very accurately the energy exchange by indirect methods.

The study of the composition of the urine, particularly the relation of the output of nitrogen to the intake in the food, is essential. When great exactitude is required the output of total nitrogen in the fæces must also be determined. A small amount of nitrogen is also excreted in the sweat, also by the loss and growth of epithelial structures, but as a general rule this loss is neglected. Since the average protein contains approximately 16 per cent. of nitrogen each gramme of nitrogen excreted corresponds to 6.25 gms. of protein.

The end products of the metabolism of fats and carbohydrates under normal conditions are water and carbon dioxide. As it is technically very difficult to estimate water-gain and loss the gaseous component is selected. We can, it is true, gain a good deal of information about the changes in metabolism by the study of the carbon dioxide output, but if we wish the fullest possible information about the nature of the changes, not merely the combustion, but the energy output, we must also determine at the same time the oxygen consumption.

This combined method is carried out most easily by the use of the Douglas bag, where by means of a mouthpiece or face mask with two

Foodstuff.	Percentage of					Calorific Value per oz.
	Water.	Salts.	Protein.	Carbo- hydrate	Fat.	
Beef (average) :						
Forequarter . . . . .	62.3	0.93	18.8	—	18.4	70.4
Hindquarter . . . . .	50.8	0.82	16.6	—	29.9	98.0
Brisket . . . . .	50.6	0.74	15.7	—	32.0	102.6
Steak . . . . .	43.9	0.81	13.7	—	41.5	125.4
Tinned Meat . . . . .	67.2	1.5	24.0	—	7.1	46.6
Veal . . . . .	71.4	1.2	19.6	—	3.6	32.2
Mutton (leg with bone) . . . . .	51.7	0.82	16.1	—	28.5	93.8
Lamb (ditto) . . . . .	58.6	1.01	17.2	—	20.7	74.5
Pork (ditto) . . . . .	50.5	0.7	16.3	—	11.9	78.9
Bacon (streaky) . . . . .	27.0	7.8	10.3	—	54.2	154.9
Ham . . . . .	31.0	4.4	10.7	—	40.5	119.0
Eggs (without shell) . . . . .	73.7	1.1	12.3	—	11.3	45.9
Eggs (dried, average) . . . . .	6.6	3.9	40.3	—	39.6	162.3
Milk (fresh) . . . . .	87.6	0.7	3.3	4.8	3.6	18.9
Milk (dried, skim, average) . . . . .	4.4	6.3	26.5	45.0	12.0	114.9
Milk (dried, whole, average) . . . . .	5.2	5.9	24.5	35.1	24.1	132.9
Cheese (Cheddar) . . . . .	33.7	4.4	25.2	—	33.4	121.2
Butter . . . . .	13.9	0.4	0.4	—	84.3	222.7
Margarine (average) . . . . .	13.0	1.6	0.2	—	84.8	223.5
Herring (fresh) . . . . .	48.3	1.7	14.5	—	10.4	44.2
Kipper . . . . .	48.4	3.7	15.6	—	14.4	56.1
Whiting . . . . .	51.1	0.7	11.3	—	0.1	13.3
Haddock . . . . .	51.6	0.9	12.0	—	0.2	14.5
Cod . . . . .	70.3	1.1	15.7	—	0.1	18.5
Potatoes . . . . .	76.2	1.3	1.9	19.8	0.04	25.4
Carrots . . . . .	67.8	0.9	1.2	9.0	0.08	12.1
Turnips . . . . .	81.3	0.6	1.1	3.8	0.09	5.9
Peas (dried split yellow) . . . . .	12.6	2.4	20.3	63.0	0.7	98.7
Beans (small haricot) . . . . .	11.1	3.5	17.8	64.5	0.5	97.0
Lentils . . . . .	12.3	2.1	20.1	63.9	0.4	98.7
Barley (pot) . . . . .	10.5	1.1	7.0	79.9	0.8	103.1
Oatmeal (medium) . . . . .	6.9	1.8	11.9	70.0	8.6	117.9
Rice . . . . .	12.2	0.8	5.9	80.3	0.4	101.2
Flour . . . . .	11.3	0.5	9.6	77.7	0.8	103.6
Bread . . . . .	42.9	1.1	7.0	48.3	0.7	66.0
Sugar (cane) . . . . .	—	—	—	100.0	—	112.2

one-way valves *all* the expired air is collected. At the end of the period of collection the air in the bag is carefully measured by means of an accurately calibrated meter (a sample being drawn off for analysis during the process of measurement), note being taken of the temperature and the barometric pressure. The sample of air is analysed in the Haldane gas analysis apparatus and the percentage of oxygen and carbon dioxide present determined.

The total volume of carbon dioxide excreted and of oxygen taken in has now to be calculated at 0° C. and 760 mm. pressure. As the volume of expired air has been measured moist it is necessary to ascertain its volume when dry. This calculation is rendered easy by the use of a table devised by Haldane. If an example is taken explanation will be easier. Let it be assumed that analysis showed the sample of

expired air to contain 3.54 per cent.  $\text{CO}_2$  and 16.86 per cent.  $\text{O}_2$ , that the *inspired* air contained 0.03 per cent.  $\text{CO}_2$  and 20.93 per cent. oxygen, and that the total volume of air expired was 99.75 litres. The corrected volume was 90.96 litres. Then the amount of carbon dioxide expired was at 0° and 760 mm.

$$\frac{3.54 - 0.03}{100} \times 90.96 = 3.192 \text{ litres.}$$

In order to obtain the true amount of oxygen taken in, the "apparent" respiratory quotient is first obtained.

$$\frac{3.54 - 0.03}{20.93 - 16.86} = 0.862.$$

This is not the true respiratory quotient as the volume of nitrogen present has altered in relation to the oxygen. Haldane has devised the following table which gives the true R.Q. from the apparent :

Apparent R.Q.	0	1	2	3	4	5	6	7	8	9
0.7	0.649	0.660	0.671	0.682	0.693	0.704	0.715	0.726	0.738	0.749
0.8	0.760	0.771	0.783	0.794	0.806	0.817	0.829	0.841	0.853	0.865
0.9	0.877	0.889	0.901	0.913	0.925	0.937	0.950	0.962	0.975	0.987
1.0	1.00	—	—	—	—	—	—	—	—	—

The apparent respiratory quotient 0.86 is then from this table 0.83. If the volume of carbon dioxide given off is divided by this figure, the true respiratory quotient, the volume of oxygen absorbed is obtained. In the example given then  $3.192 \div 0.83 = 3.840$  litres of oxygen. As the duration of the experiment was ten minutes it follows that the oxygen consumption was at the rate of 384 c.c. per minute.

The relative combustion of carbohydrate and fat can be calculated from the respiratory quotient as, the experiments being of short duration, it is assumed that during the actual period of the experiment only carbohydrate and fat are utilised. If the nature of the experiment demand it, the protein consumption can be determined from the output of nitrogen in the urine. For every gramme of urinary nitrogen 8.45 gm. oxygen (1 gm.  $\text{O}_2 = 0.699$  litre) are required for the oxidation processes and 9.35 gms. of carbon dioxide (1 gm.  $\text{CO}_2 = 0.5087$  litre) are given off. If then the appropriate amounts of oxygen and carbon dioxide be deducted from the total amount of oxygen taken in and of carbon dioxide exhaled a non-protein R.Q. is thus obtained and from this an accurate estimate of the material utilised in a given time can be made.

The fact must, however, never be forgotten that the R.Q. is a ratio and nothing more, and moreover it is a ratio of many components.

Not only can the relative combustions be determined but a caloric value for each litre of oxygen taken in has been worked out. The following table from Zuntz and Loewy summarises these results :



FOR EACH LITRE OF O<sub>2</sub> CONSUMED.

R.Q.	Caloric Value. Cals.	Utilisation of Glycogen.	Utilisation of Fat.	Loss of Body Weight.
0.7133	4.7950	g. 0.0000	g. 0.5027	g. 0.503
0.72	4.8015	—	—	—
0.75	4.8290	0.1543	0.4384	1.056
0.80	4.8748	0.3650	0.3507	1.811
0.85	4.9207	0.5756	0.2630	2.565
0.90	4.9665	0.7861	0.1753	3.320
0.95	5.0123	0.9966	0.0877	4.074
1.00	5.0581	1.2071	0.0000	4.828

Therefore with a non-protein respiratory quotient of 0.83 an intake of 384 c.c. oxygen works out in calories as  $0.384 \times 4.9023 = 1.88$  cal. per minute.

It is obvious that these results are only of value in relation to the subject on whom the experiment was done, therefore, to make the results of more general value, they must be related to some standard. The standard at present in vogue is that of the superficial area in square metres. The formula for obtaining this value has been determined by D. and E. F. Du Bois. It is :

$$\text{Surface area} = W^{0.425} \times H^{0.725} \times 71.84.$$

In order to expedite calculation they devised a most ingenious chart (Fig. 206).

If the amount of external muscular work done is known it can also be converted from kilogrammetres, the usual method of assessment, into calories. 427 kgm. are equal to 1 Calorie.

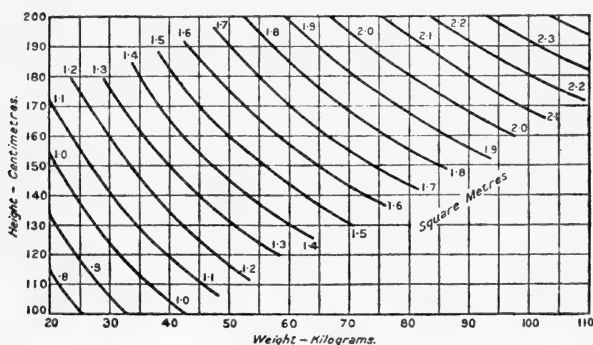


FIG. 206.

## CHAPTER XXV

## HYDROGEN ION CONCENTRATION

For the proper action of the tissue cells a balance is maintained between the acids and bases present in the tissue fluids so that they are kept constantly just on the alkaline side of neutrality. This balance is maintained in spite of the varying production of various acids during the course of metabolism, even when as the result of various stimuli, acids, such as lactic acid, are produced in excess. Acidosis does not mean that the reaction of the blood has become acid. In this maintenance of neutrality sodium carbonates and phosphates play the chief rôle; they act as "buffers" in that when they are present in solution the addition of acid has but little effect so far as the reaction of the fluid is concerned.

The real acidity or alkalinity of a solution cannot be determined by titration but by the hydrogen ion concentration ( $cH$ ).

This value varies with the concentration and the degree of dissociation of the acid or alkali tested. Thus a decinormal solution of HCl as commonly used in titrations is not 0.1 normal, but only about 0.09 normal when valued in terms of hydrogen ion concentration. This is due to the fact that dissociation of HCl into  $H^+$  and  $Cl^-$  is not complete. The same is true of alkaline solutions which may be expressed in terms of diminishing hydrogen ion concentration instead of increasing hydroxyl ion concentration. The neutral point for all standards is taken as that of specially distilled (pure conductivity) water, where  $[H^+] = [OH^-]$  and the H ion value is  $10^{-7}$ . The various values are now usually stated in terms  $pH$ , which is the logarithm of the hydrogen ion concentration ( $cH$ ) with the minus sign omitted,  $cH$  of  $10^{-7} = pH$  of 7.

Note that as the  $pH$  value decreases the hydrogen ion concentration increases, the solution becomes more acid and as it increases the solution becomes more alkaline.

The approximate  $pH$  values of various secretions, etc., are given in the following table:

	$pH$
Gastric juice . . . . .	1.77
Urine . . . . .	5.5-7
Milk (cow) . . . . .	6.6
" (human) . . . . .	7.0
Saliva . . . . .	6.9
Blood . . . . .	7.4
Fæces (variable) . . . . .	8.0
Pancreatic juice . . . . .	8.3

Two methods have been employed for the determination of the hydrogen ion concentration, (a) the electrometric method and (b) the indicator or colorimetric method. The first of these methods demands the use of very elaborate apparatus, whereas the second, while perhaps not quite so accurate, is more easily carried out.

Whatever the nature of the process certain chemical bodies called indicators change in colour with variations in the hydrogen ion concentration. In recent years a large number of indicators have been introduced which permit of the determination of a wide range of reaction ( $pH$  of 1 to  $pH$  of 14) with ease and rapidity.

In carrying out the determination an ordinary test tube rack, with a

sheet of white paper pinned behind it, when tilted slightly backwards serves quite well. For more accurate work a colorimeter or a blackened box called a comparator may be made use of.

The indicators commonly employed are as follows :

Solution. ]	Indicator.	pH Range.	Colour. Acid→Alkaline.
0.04% in water	Thymol blue (acid range)	1.2- 2.8	Red→Yellow
0.1% in 50% alcohol	Methyl orange . .	3.1- 4.4	Red→Yellow
0.04% in water	Brom-phenol blue	3.0- 4.6	Yellow→Blue
0.02% in 60% alcohol	Methyl red . . .	4.4- 6.0	Red→Yellow
0.04% in water	Brom-cresol purple	5.2- 6.8	Yellow→Purple
0.04% in water	Brom-thymol blue.	6.0- 7.6	Yellow→Blue
0.02% in water	Phenol red . . .	6.8- 8.4	Yellow→Red
As above	Thymol blue (alkaline range)	8.0- 9.6	Yellow→Blue
0.05% in 50% alcohol	Phenol-phthalein .	8.3-10	Colourless→Red
0.04% in 50% alcohol	Thymol-phthalein	9.3-10.5	Colourless→Blue

The amount of indicator added is important. Speaking generally, 5 drops of any of the foregoing solutions added to 10 c.c. of the solution to be tested give a good result.

A series of standard solutions may be prepared with different pH values such as the convenient standards of Clark and Lubs. (An excellent account of the methods, indicators and various standard solutions and their preparation is to be found in *The Determination of Hydrogen Ions*, by W. M. Clark, Baltimore, 1920.) The various salts required for the preparation of the standard solutions can now be purchased in a pure state.

A range of pH from 1-3 may be obtained with 0.1 N HCl (1.04), 0.02 N HCl (1.72), 0.1 N acetic acid (2.89).

A range of pH from about 3.5-6 may be obtained by combining various volumes of 0.1 N acetic acid and 0.1 N sodium acetate.

A range of pH from 6-8, the most important physiological range, is best prepared from standard Sørensen phosphate solutions. (1) An  $\frac{M}{15}$  solution of *primary* potassium phosphate which contains 9.078 gms.

$KH_2PO_4$  per litre, and (2) an  $\frac{M}{15}$  solution of *secondary* sodium phosphate which contains 11.876 gms.  $Na_2HPO_4 \cdot 2H_2O$  per litre.

Brom-thymol blue and phenol red are satisfactory indicators at this range of pH concentrations.

In order to test the turning point of the various indicators at varying hydrogen ion concentration measure 5-7 c.c. of distilled water into a clean beaker, add 5 drops of one of the indicators, and then run in carefully from a burette 1 c.c. of decinormal sodium hydrate. Note the colour. Then add from another burette, drop by drop, decinormal acetic acid until the colour of the indicator changes. Note the colour and the amount of acid required. Repeat this (a) with the whole series of indicators, and (b) using decinormal hydrochloric acid in place of the acetic acid.

## PHOSPHATE MIXTURES.

Secondary.	Primary.	pH.
c.c.	c.c.	
1.0	9.0	5.906
2.0	8.0	6.239
3.0	7.0	6.468
4.0	6.0	6.643
5.0	5.0	6.813
6.0	4.0	6.979
7.0	3.0	7.168
8.0	2.0	7.381
9.0	1.0	7.731
9.5	0.5	8.043

To test the "buffer" action of salts like sodium bicarbonate, compare the amounts of decinormal hydrochloric acid which must be added to obtain the turning point of an indicator such as methyl orange, (a) using 10 c.c. distilled water and (b) 10 c.c. of a 0.25 per cent. solution of sodium bicarbonate. Repeat this experiment with solutions of phosphates, citrates and acetates.

A similar type of experiment may be done, using bromthymol blue or phenol red as indicator, by breathing through (one full expiration) (a) 0.25 per cent. sodium chloride, (b) 0.25 N, (c) 0.025 N, and (d) 0.0025 N sodium bicarbonate solutions.

Standard tubes of the various solutions containing identical amounts of the indicators (5 drops) should be prepared for comparison. Try experiment with each indicator.

Similar tests may be made of the reaction of the blood, but the dialysis method introduced by Dale and Evans (*Jour. of Physiol.*, 1920, 54, 167) gives much more accurate results.

# APPENDIX

## ANALYTICAL TABLES

### (OUTLINE OF METHOD FOR DETECTION OF VARIOUS SUBSTANCES IN A MIXTURE.)

The following Physical Properties should be noted :

#### I. Appearance.

A. *Powder*.—Dust some on to a slide and examine under the microscope for starch grains and crystals. Dissolve some in a suitable solvent.

B. *Solution*.

1. **Opaque**—may be due to :

- (a) suspended fat globules—clear up with ether ;
- (b) certain inorganic salts—clear up with mineral acid ;
- (c) certain proteins.

2. **Opalescent**—may be due to :

- (a) glycogen or starch—iodine reaction ;
- (b) certain proteins.

3. **Deeply coloured**—suspect blood.

#### II. Reaction.

A. *Acid*—may be due to :

- (a) free acid
  - (b) acid salt
- } apply congo red test.

If due to free acid, ascertain whether this be

- 1. a mineral acid or
  - 2. an organic acid
- } apply Gunzberg's test.

If due to an organic acid, apply Uffelmann's test for lactic acid.

B. *Alkaline* test for carbonic acid (effervescence with mineral acid), ammonia (smell, etc.), caustic alkali.

The following Chemical Tests should now be applied to suitable quantities of the solution.

#### I. For Carbohydrates.

1. Apply *Trommer's test*.

A. **Positive**—indicates **monosaccharides, lactose, or maltose**.

B. **Negative**, but complete solution of cupric hydrate obtained on adding caustic alkali, indicates **cane sugar**. Confirm for this by boiling some of the solution with a mineral acid for a minute or so, and applying Trommer's test

to the product—reduction indicates cane sugar. The original solution will also taste sweet.

**C. Negative**, and no solution of cupric hydrate. Absence of monosaccharides and disaccharides.

2. **Add Iodine Solution.**

(a) A blue colour which disappears on heating, and returns on cooling indicates *starch*.

(b) A brown colour which disappears on heating and returns on cooling indicates *dextrin* or *glycogen*. Confirm for polysaccharides by heating some of the original fluid for about fifteen minutes with a mineral acid, and testing for *sugar* in the hydrolysed fluid.

**To distinguish between Starch, Glycogen, and Dextrin.**—Shake up some of the original powder with *cold* water and filter. By this treatment glycogen and dextrin will dissolve, starch will not. Wash the filter paper thoroughly with water, then add a drop of iodine solution—a blue stain indicates starch. Add iodine solution to the filtrate—a red colour indicates dextrin or glycogen; if the former body be present the filtrate is clear, opalescent if the latter.

**To distinguish between Dextrose, Maltose, and Lactose.**

(1) Prepare *osazone crystals* and examine under the microscope—dextrosazone gives long thin needles; maltosazone, short thick needles; lactosazone, needles of varying length and thickness.

(2) *Barfoed's reaction* may also be tried.

II. **For Proteins.**

1. Apply the *Biuret reaction*—(a) A violet colour indicates native proteins; (b) a rose pink colour, proteose or peptone.

2. Apply *Millon's* and the *Xantho-proteic* tests.

(a) A well-marked reaction indicates ordinary proteins

(b) A faint reaction (combined with a distinct Biuret, and the absence of coagulation on boiling) points to **gelatine**. (Confirm by noting if the solution gelatinises on cooling.)

**If the Biuret Test gives a Violet Coloration,**

**A.** Add a drop or so of dilute acetic acid and boil. A coagulum points to **native proteins**. To ascertain which of these is present (i.e. albumin or globulin), half saturate some of the solution with  $(\text{NH}_4)_2\text{SO}_4$ . A precipitate indicates **globulin**; filter; if the filtrate still gives a coagulum on boiling, **albumin** is present.

**B.** Carefully neutralise some of the solution. A precipitate may be:

- |  |   |   |
|--|---|---|
| 1. <b>Alkali Meta-protein</b> —original fluid alkaline | } | the precipitate re-dissolves on adding excess of acid or alkali.    |
| 2. <b>Acid Meta-protein</b> —original fluid acid       |   |   |
| 3. <b>Nucleo-protein</b> —original fluid alkaline      | } | precipitate does not disappear on adding a moderate excess of acid. |
| 4. <b>Mucin</b> —original fluid alkaline               |   |   |

**To distinguish between Nucleo-protein and Mucin.**—This is possible only when a large amount of these bodies is present. The acetic acid precipitate is collected on a filter paper, washed with acidulated water, and divided into two portions *a* and *b*.

- (a) Boil with 20 per cent. HCl for 10 minutes; cool; neutralise; apply Trommer's test. A positive reaction points to *mucin*.
- (b) Melt in a crucible with fusion mixture; after the ash cools, dissolve it in nitric acid and add molybdate of ammonia solution. A yellow precipitate on warming indicates **Nuclein**.

**If the Biuret Test gives a Rose Pink Coloration**, add a few drops of concentrated pure nitric acid.

A. A *white precipitate*, which clears up on warming and returns on cooling, points to **Proteose**. Confirm by the salicyl sulphonic acid test.

If proteose be present, saturate some of the original fluid, from which native proteins have been separated by boiling with sodium chloride. A precipitate indicates **primary proteoses**. Filter and add a drop of acetic acid; a precipitate points to **secondary proteoses**.

B. *No precipitate* with nitric acid, but a distinct pink Biuret reaction, points to **Peptone**. Confirm by saturating the original fluid with ammonium sulphate, filtering and applying the Biuret test to the filtrate.

**When two or more Proteins are present**, the following method will be found very useful.

Add a few drops of salicyl sulphonic acid to several c.c. of the original fluid. A white precipitate may indicate native protein or proteoses. Boil. The proteoses dissolve, whereas the native protein becomes coagulated. Filter hot. If a precipitate forms in the filtrate on cooling it indicates **Proteoses**. Filter off this precipitate and apply the Biuret test to the filtrate. A rose pink coloration indicates **Peptone**.

III. **For Fats.**—In watery solution fat may be dissolved as a *soap*. The presence of this can be detected by pouring some of the original fluid into about 20 c.c. of 20 per cent.  $H_2SO_4$  contained in a small beaker, and heated to near boiling point. If soap be present a film of fatty acid will form on the surface of the fluid.

IV. **The following substances should also be tested for.** I. **Bile salts**—Pettenkofer's reaction; II. **Bile Pigments**—Gmelin's test.

V. **Urea.**—(1) Add some fuming nitric acid to some of the original fluid. Effervescence points to urea.

(2) Repeat with hypobromite solution.

(3) If 1 and 2 be positive, confirm by obtaining urea nitrate crystals. To do this evaporate about 30 c.c. of the original fluid to small bulk, extract residue with six times its bulk of methylated spirit, evaporate this extract to dryness, dissolve residue in 3-4 c.c. distilled water, and add to the resulting fluid a few c.c. of *pure* nitric acid, meanwhile keeping the test-tube cool by holding it under the tap. Crystals of urea nitrate separate out if urea is present. Examine under microscope.

VI. **Uric Acid.**—Apply murexide test.

VII. **Blood Pigment.**—(1) Examine by means of the spectroscope. *A*, the original fluid; *B*, the same after reduction; *C*, the same after the addition of caustic alkali and heating. By this latter method alkali hæmatin is formed. This itself does not give a very distinct absorption band, but if a reducing agent ( $\text{NH}_4\text{HS}$ ) be added to it hæmochromogen is formed, which has two very distinctly marked bands in about the same position as those of oxyhæmoglobin.

(2) Apply the guaiac and ozonic ether test.

When it is desired to ascertain whether Ferments be present it is necessary to add a piece of coagulated egg-white, or of washed fibrin, to the original fluid, and to place the mixture on a waterbath heated to body temperature. If, after an hour, the digest gives a distinct proteose reaction, and this was not obtained in the original fluid, the presence of a proteolytic ferment may be assumed; **pepsin**, if the original fluid react acid, and **trypsin**, if it react alkaline. If proteoses are present in the fluid itself, some method like Mett's must be employed to identify the ferment.

For the detection of **Diastatic** and **Lipolytic** ferments, the methods described in the text must be employed.

For the detection of the various substances which may occur in the urine, the test and reactions described in the text must be applied.

---

NITROGEN FACTORS FOR CERTAIN ORDINARY METABOLIC PRODUCTS  
FOUND IN URINE.

Urea	= N × 2·145
Ammonia	= N × 1·214
Uric Acid	= N × 3·00
Creatinine	= N × 2·695
Creatine	= N × 3·12



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