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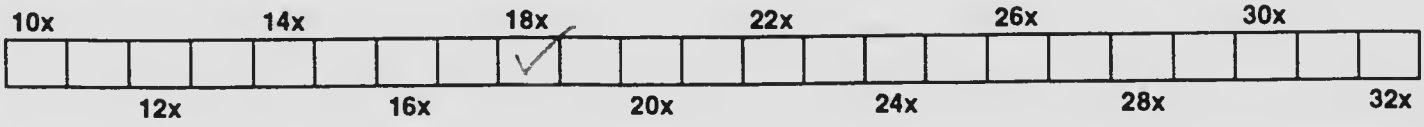

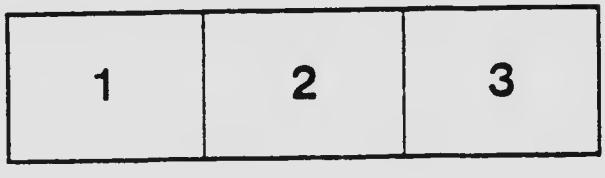

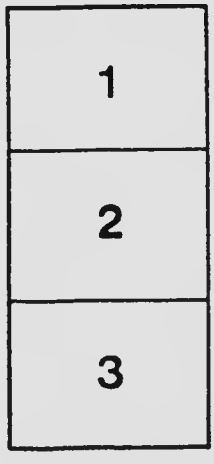

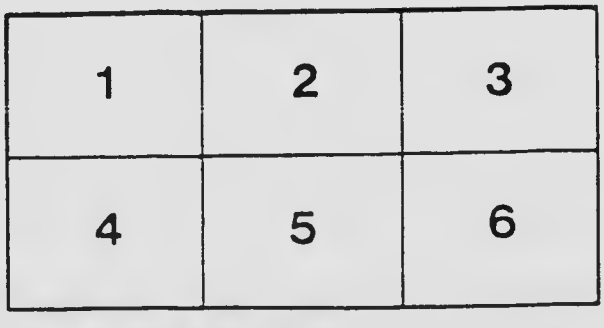

CLASS

LABORATORY EXERCISES

—IN—

PHYSIOLOGY

BY

WESLEY MILLS, M.A., M.D.,

Professor of Physiology in McGill University.

MONTREAL.

C. ASHFORD, PUBLISHER,

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CLASS LABORATORY EXERCISES.

FOOD STUFFS, ARTIFICIAL DIGESTION, ANIMAL FLUIDS, ETC.

I. Starch, Dextrin, Dextrose, Fats, Soaps.

1. **Starch.** Insoluble in cold water; dissolves imperfectly to an opalescent liquid with heat.

2. The latter cooled gives a blue color with solution of iodine.

3. Heat gently in test tube till the color *begins* to disappear, then immerse the tube in cold water; the color will return.

4. **Dextrin.** Soluble in water; the solution gives a red brown color with solution of iodine.

5. Treat as Starch (in 3); the color vanishes and reappears. This test requires plenty of iodine

6. **Dextrose.** *(Grape Sugar)* crystalline, readily soluble in water, less so than cane sugar; reduces metallic oxides as in the following test.

7. *Trommer's Test:* To a couple of drops of solution (10%) of $Cu\ S\ O_4$ add a small quantity of the solution of sugar, then add K OH (or Na O H) till a clear blue solution results, and heat gradually to boiling Boil for half a minute and let stand. Note the various changes—yellow or red precipitates.

Reactions: (1) $Cu\ S\ O_4 + 2\ (K\ O\ H) = Cu\ O + H_2\ O + K_2\ S\ O_4$.

(2) Cu O is *reduced* by sugar to $Cu_2\ O$.

The light yellow that first appears is the hydrated $Cu_2\ O$.

8. *Moore's test:* Heat a solution of sugar with solution of K O H; the color changes to a shade of brown, the depth depending on the strength of the saccharine solution.

9. *Bismuth* (Böttger's *test*: Heat a solution of sugar with a pinch of bismuth subnitrate and K O H; a brown color and dark precipitate (on standing) results.

11. *Fermentation test*: A solution of sugar with a little yeast added placed in a test tube and kept in a beaker of water at about 70° C. gives off $C\ O_2$ and forms alcohol.

Reaction: $C_6\ H_{12}\ O_6 = 2\ (C_2\ H_5.\ O\ H) + 2\ C\ O_2$.

12. *Conversion of starch into dextrose:*

To dilute solution of starch add a few drops of $H_2\ S\ O_4$ and boil for 5–10 minutes.

The starch solution becomes limpid.

Test for sugar by Trommer's method adding excess of K O H to neutralize the acid.

The liquid contains also dextrin and unaltered starch.

13. *Glycogen:* throw a piece of fresh liver into a little boiling water in a beaker; add a few drops of acetic acid. After boiling for one minute empty the beaker into a porcelain capsule and break up the liver into small pieces. Return once more to the beaker and boil for two minutes. Filter into a clean test-tube. The filtrate may be expected to contain glycogen. When cool test as follows: (a) Note milky appearance peculiar to solutions of glycogen. (b) Add iodine and note the port wine or reddish brown colour.

14. *Fats.* Insoluble in water; soluble in ether, hot alcohol, chloroform, benzol, carbon bisulphide, turpentine, etc.; lighter than water. Test solubillty in water and ether.

15. Boil a little butter or lard in a solution of K O H in a beaker or porcelain capsule, for some time. A soap is formed; test by shaking up with soft water and note that solution is complete and soap suds formed.

To some of the potash soap add a little Ca Cl_2 in solution; a calcium soap (insoluble) is precipitated. Repeat with Mg S O_4.

16. Add to the potash soap in a test tube a little 25% H_2 S O_4 and boil; on standing a layer of fatty acid rises to the top; it smells of butter.

Tests: (a) Pour over paper a little ethereal solution of fat; on the evaporation of the ether a characteristic stain remains.

(b) Pour a little ether over a suspected liquid (*e g.*, soap solution); rapidly filter into a clean, dry watch glass or test tube; evaporate; if fat is present it will remain on the glass. This test applies also to fatty acids.

Reactions:

Palmitin—a fat. Glycerine. Potassium. Palmitate—a soap,

$$C_3H_5(C_{16}H_{31}O_2)_3 + KOH = C_3H_5(OH)_3 + 3\,KC_{16}H_{31}O_2$$

Potassium palmitate. Palmitic acid—a fatty acid.

$$2\,K\,C_{16}H_{31}O_2 + H_2SO_4 = 2\,C_{16}H_{32}O_2 + K_2SO$$

17. Emulsification *(a)* Place on a glass slide a few drops of 1% sodium carbonate solution and let a drop of oil fall in the middle. The oil drop soon shows a white rim and a white milky opacity extends over the solution. *(b)* Shake up a few drops of oil with

(1) Water.
(2) 1% sodium carbonate solution.
(3) Gum arabic solution.

Inferences from the experiments of exercise I:

Starch is insoluble in cold water; imperfectly soluble in hot water; gives a blue color-reaction with iodine; may be converted into dextrin and dextrose.

Dextrin is soluble in water; gives a red color-reaction with iodine.

Glycogen forms a milky or opalescent solution; gives a red brown colour with iodine.

Dextrose is soluble in water; reduces metallic oxides.

Fats which are compounds of glycerine and a fatty acid (ethereal salts) may be decomposed by a strong alkali, with the formation of soaps; are insoluble in water, etc.

Some *Soaps* are soluble in water (alkaline) and others insoluble; by heating with a strong mineral acid may be decomposed liberating free fatty acid.

Oil will not mix with water but will form an emulsion with a solution of an alkali or with a viscid liquid.

II. Proteids: Albuminous Substances.

Preparation of Proteid Solution: Cut up white of egg with scissors; dilute 7–10 times with water; filter through linen and afterwards through paper. This filtrate contains albumin and globulin.

Tests for Proteids.

1. Boil a little of the solution in a test tube and add a drop of acetic acid; it coagulates, showing that albumin or globulin is present.

2. Treat the *coagulum* with strong mineral acids (H Cl, H_2 S O_4) it does not dissolve readily.

3. Add to a similar portion of the original solution strong H N O_3. It coagulates, showing presence of albumin or globulin.

4. Add to a portion of the solution some nitric acid and boil; a yellow color is developed. Cool and add N H_4 O H: the yellow changes to orange. This is known as the *Xanthoproteic reaction.* It is given by all proteids, as are the three following tests.

5. Add to another portion a drop of *Millon's reagent.* The resulting precipitate turns red on boiling. Millon's reagent is Hg dissolved in its own weight of strong H N O_3 and then diluted with twice its volume of water.

6. Add to another portion potassium ferrocyanide and acetic acid. A white precipitate falls.

7. *Piotrowski's Reaction:* To another portion add a few drops of a solution prepared by adding one part of Cu S O_4 solution to 10 parts of water, and some solution of K O H. A violet color appears.

8. *Biuret Reaction:* If the amount of $CuSO_4$ be very small, proteoses and peptones give a rose color instead of a violet.

9. Prepare some dilute acid and alkali by adding one drop each of hydrochloric acid and sodium hydrate, to test tubes ¼ full of water.

Acid Albumin. Add to a little of the solution of albumin an equal bulk of dilute acid, gently warm for some time in a water bath; *acid albumin* is formed; ascertain that it does not coagulate on boiling by testing a small portion. Show that it is precipitated by neutralization as follows: To a portion of the acid albumin solution add some sugar to increase the specific gravity and pour carefully on top of it some dilute alkali. Note the cloudy ring at the point of junction, neutral zone. The addition of a few drops of litmus improves the above test.

Alkali-Albumin. Add to a similar portion of albumin a few drops of diluted alkali (KOH or NaOH) and gently warm as before; *alkali-albumin* is formed. Apply the boiling test. Neutralize with acid, using the same precautions as for acid albumin.

10. **Coagulation Point.** Use three test tubes with contents as follows:

(a) Solution of albumin alone.

(b) Solution of albumin with a drop of ·02% acid.

(c) Solution of albumin with a drop of ·02% alkali.

Heat gradually in a water bath with a thermometer and note the coagulating point of each. In these heating tests we are dealing with ordinary albumin as the acid and alkali are not strong enough to effect the conversion to acid and alkali albumin.

Inferences: Coagulated albumin is insoluble in acids except after prolonged action; *acid-albumin* and *alkali-albumin* are not coagulated by boiling; are insoluble in neutral liquids (water), but soluble in acids and alkalis.

III. FOODSTUFFS.

MILK, FLOUR, BREAD.

These furnish specimens of all the essentials of foods, organic and inorganic.

1. **Milk** (cow's). Reaction alkaline neutral or amphoteric. ($H_2 Na P O_4$, $H Na_2 P O_4$). Specific gravity 1028 to 1034.

Skimmed milk has a *higher* S. G.

(a) **Proteids.** Place a little milk in a test tube, add a few drops of acetic or dilute sulphuric (25%) acid; gently warm a little above blood heat; a granular precipitation of caseinogen (with fat) will take place. Let stand.

(b) To the rest of the milk in the beaker add some extract of rennet, warm without shaking to 37° C. A gelatinous firm clot forms consisting of casein.

(c) Filter both and test the filtrate (whey) for milk sugar (Trommer's test) and for lactalbumin (boiling).

(d) After precipitating the lactalbumin in *c* by boiling, filter it off and test the filtrate for salts (chlorides) with solution of silver nitrate.

(e) Test the curd remaining on the filter in *b* for entangled fat (butter) as in Chapter 1, 16, *b*.

2. **Flour.** Wash a dessert-spoonful of flour through a piece of fine muslin held as a bag.

Test wash water for starch, and sugar (little or none).

The remnant of the washing is *gluten*. It is very tenacious. It gives proteid reactions (Millon's reagent, II, 5).

3. **Bread.** Soak in a capsule with warm water. Filter. Use as little water as possible.

Test the filtrate for starch and sugar.

Test residue for proteid (Millon's reagent) and starch.

Conclusions: Milk contains all the essentials of a foodstuff (fats, carbohydrates, proteids and salts); the "particulate" caseinogen and globular fat may be precipitated by chemical treatment; the salts are in solution in the water of milk.

Flour contains starch and proteid.

Bread, owing to the heat used in cooking, has had part of its original starch converted into dextrose.

IV. Artificial Peptic Digestion,

1. An artificial gastric juice may be made by extracting minced or scraped mucous membrane of the (pig's) stomach with 0·2% H Cl, or by extracting the same with glycerine, or by dissolving commercial pepsin in 0·2% H Cl, which may be made by adding a drop of strong H Cl to a test tube full of water.

2. Test artificial gastric juice for peptone (Biuret reaction). Peptone may be found owing to (self) digestion of the mucous membrane.

3. Use six test tubes with contents as follows:

(a) One quarter of a test tube full of 0·2% H Cl and small pieces of washed fibrin. The fibrin must be teased up into very fine shreds.

(b) One quarter of a test tube full of 0·2% H Cl and pepsin, with fibrin.

(c) One quarter of a test tube full of water with pepsin and fibrin.

(d) One quarter of a test tube full of 1% sodium carbonate solution with pepsin and fibrin.

(e) Prepare a tube like *b*, but boil it and cool it down again before adding the fibrin.

(f) Prepare another tube like *b*.

4. Put *f* in the test tube rack to ascertain whether digestion will take place in the cold. Place all the others appropriately labeled in a beaker containing water and keep for one hour at 37° C.

At the end of fifteen minutes test each for acid albumin (see Lesson II, 9).

At the end of one hour test each for peptone and for propeptone (albumoses, proteoses.)

Note especially the changes in the naked eye appearances of the fibrin at frequent intervals.

Test for Peptones: With caustic alkali and a *trace* of $CuSO_4$ they give rose color (Biuret reaction) They are not precipitated by saturation with ammonium sulphate.

Test for Propeptones: Add HNO_3. A precipitate results, which disappears on heating, but reappears on cooling.

Conclusions: Proteids acted upon by pepsin in a medium of very dilute HCl at blood heat yield acid-albumin, proteose and peptone.

V. Salivary and Pancreatic Digestion.

Salivary Digestion.

1. Prepare some gelatinous starch and let it cool.

2. Test it for dextrose. If it contains none it is suitable.

3. Place some of the starch solution in a test tube and add a little saliva; shake up, add a little of Trommer's sugar-test liquid and at once gradually heat up to boiling. The disgestive action is often rapid enough to form sugar even under such circumstances.

4. Let another tube with starch and saliva stand in a water bath (beaker) for 5–10 minutes at 37° C. Abundance of sugar will be found on testing.

5. Place some saliva in a test tube and boil; after cooling, add starch and place in a water bath (beaker) at 37° C. After 10 minutes test for sugar.

Pancreatic Digestion.

1. Watery extract of minced pancreas (ox, pig, etc.) will suffice; but glycerine extract is likely to contain no albumin, and the merest trace, if any, of peptone. Commercial pancreatin may also be used.

The best menstruum is solution of $Na_2 C O_3$ (1%).

1. Place in water bath tubes with the following contents:

(a) Glycerine extract of pancreas or pancreatin, sodium carbonate solution, and a very little teased up fibrin.

(b) Another tube with the same contents as *(a)* that have been boiled and allowed to cool.

(c) As *a*, but with 0.2% H Cl instead of $Na_2 C O_3$.

(d) As *a*, but with water instead of $Na_2 C O_3$.

Note changes in appearance and test each tube for *peptone* and *a* for *alkali-albumin*, albumose and peptone.

3. Test as in the case of saliva, the *amylolytic* action of glycerine extract of pancreas.

(a) With $Na_2 C O_3$ menstruum. *(b)* Without it.

Note—If the pancreatin used gives the test for sugar as it sometimes does, the digestion of the starch may be demonstrated by testing from time to time with iodine. When the mixture no longer gives a blue colour digestion has taken place. For this method to be successful the starch solution must be very dilute.

4. For the demonstration of the action of pancreatic juice on fats a fresh extract is required, as steapsin is not present in glycerine extracts.

(a) Rub up a little minced pancreas in a mortar; digest for some time with warm water; filter; neutralize the filtrate if necessary with solution of $Na_2 C O_3$.

(b) Rub up in small mortar or porcelain capsule a little olive oil with 3–5 times its volume of the watery extract *a*. A creamy emulsion will form. Let it stand.

(c) Shake up a few drops of neutral olive oil or melted lard in a test tube with a little of the infusion *a;* heat slowly up to 35°–38°. Let stand for 5–10 minutes, and then place a drop from the bottom of the tube on blue litmus paper: a red stain will be produced by the fatty acid set free by the digestion of fat.

Reaction.

Palmitin. Glycerine. Palmitic acid.

$$C_3 H_5 (C_{16} H_{31} O_2)_3 + 3 H_2 O = C_3 H_5 (O H)_3 + 3 (C_{16} H_{32} O_2)$$

The *negative* action of salivary and gastric juices on fats and of gastric juice on starch may be readily shown.

The satisfactory demonstration of the presence of leucin and tyrosin in artificial pancreatic digestion requires more time and somewhat more elaborate methods than the above.

Inferences: Pancreatic digestion is most active in an alkaline medium. It results in the formation of alkali-albumin, proteoses, and peptones from proteids, fatty emulsion and fatty acids from fats, and sugar from starch.

VI. BILE.

Ox bile may be used for the following experiments :

1. Its reaction is alkaline and color greenish.

2. Acidulate a little bile with acetic acid. A precipitate of mucin (or nucleo-albumin) is obtained.

3. Filter off the precipitate obtained in 2. Boil the filtrate, no proteid coagulable by heat is present.

4. Make a solution of acid-albumin and render it *almost* neutral, then add a drop of bile.

The mixture curdles; a larger addition of bile may redissolve the precipitate.

Pettenkofer's Test for **Bile Acids** (and bile salts).

This is really a test for *cholic* acid.

(a) To a few drops of bile on a porcelain surface (capsule) add a solution of cane sugar and an excess of H_2SO_4 added gradually to develope the characteristic purple color.

It may be noted that cholic acid. is first precipitated and then dissolved.

(b) Repeat, using a test tube instead of porcelain as follows: shake up some bile and solution of cane sugar together till a froth forms on top. Pour a few drops of H_2SO_4 down the side of the tube and it will leave a purple streak through the froth.

5. **Gmelin's Test** for **Bile Pigment.**

(a) Place a few drops of bile on a porcelain surface: put 1–2 drops strong, impure nitric acid (containing nitrous acid) in the centre of the layer of bile. Zones of color will be formed in the order: *Green*, *blue*, *red violet*, *yellow*. The green is the most characteristic.

(b) Place a little nitric acid in a test tube and allow bile to run slowly down the side of the inclined tube. The colors will appear at the line of contact of the fluids.

6. Prepare a dilute solution of bile in a test tube. Drop in a little flowers of sulphur. Note that it will sink. Repeat using pure water instead of bile solution and compare.

7. *Cholesterin* is best obtained from gall-stones.

Extract pulverized gall-stones with ether in a dry vessel. Rapidly filter.

On evaporation needle-shaped crystals form. If a little alcohol be added to retard evaporation, the cholesterin crystallizes in characteristic rhombic plates.

Drop a few of the crystals into strong $H_2 S O_4$ and heat if necessary: they dissolve to a deep red color.

8. The pigment of gall-stones may be extracted with chloroform.

VII. URINE.

Urine freshly voided should be used for these experiments.

1. Note color ; test the reaction.

2. Determine the specific gravity at 15°–17° C. with the *urinometer*.

3. *Salts of the urine.*

Chlorides. If not acid, acidulate with HNO_3 and add a drop of solution of $AgNO_3$. A curdy white precipitate, soluble in NH_3, falls.

$$NaCl + AgNO_3 = AgCl + NaNO_3$$

Sulphates. Acidify with HCl and add 1–3 drops of solution of $BaCl_2$. A white precipitate of $BaSO_4$ falls. This precipitate is insoluble in any menstruum.

$$Na_2SO_4 + BaCl_2 = BaSO_2 + 2\,NaCl.$$

Phosphates. *(a)* Add to a teaspoonful of urine in a test tube about half its volume of strong HNO_3 and then a few drops of ammonium molybdate. On boiling a yellowish color develops ; on standing, a yellowish crystalline compound of ammonium molybdate with phosphoric acid precipitates. This test is given by both alkaline and earthy phosphates.

(b) Add a little of a solution of caustic alkali to urine and let stand.

To another portion add the same reagent and heat gently. The *earthy phosphates* are in each instance precipitated.

4. Nitrogenous constituents of the urine.

Uric Acid. To half a test tube full of urine add about one-twentieth of its bulk of strong H Cl and let stand for at least 48 hours. Crystals of uric acid resembling cayenne pepper granules may be seen, either floating on the top of the liquid, lying on the bottom or adhering to the sides of the tube.

The *Murexid* test for uric acid (and urates): To a very little *uric acid* or *urates* on a porcelain surface (capsule) add a drop of strong H N O_3 and heat gently to dryness; cool and add a drop of N H_3; a purple color is developed (purpurate of ammonia).

Urea. With a little urine that has been concentrated over the water bath to about one-third of its bulk, mix gradually, drop by drop, strong H N O_3; crystals of urea nitrate $\left\{ CO\begin{pmatrix} N & H_2 \\ N & H_2 \end{pmatrix} + H N O_3 \right\}$ form in characteristic glistening scales. This test is hastened by cooling the tube under the tap.

Creatinine. Mix with some urine a few drops of a solution of *sodium nitro-prusside*, then add caustic alkali, drop by drop; a red brown color results, which fades on boiling (Weyl's test). Add acetic acid to the boiling liquid, Prussian blue is formed.

Horse's Urine.

1. Compare color, reaction (alkaline carbonates), smell, etc., of horse's urine with that of man.

2. **Indican Test.** *(a)* To about a tablespoonful of urine add an equal volume of strong H Cl and cautiously, drop by drop, a dilute solution of chlorinated lime (shaking well after each addition); a color some shade between green and blue will develop (indigo).

Sometimes the addition of H Cl alone suffices, especially on standing to develop indigo. The decomposition may be thus expressed:

Indican. Indigo blue.

$$2.\ C_8\ H_6\ N\ K\ S\ O_5 + O_4 = 2\ (C_8\ H_5\ N\ O) + 2\ K\ H\ S\ O_4.$$

Dog's urine also contains much indican.

(b) Repeat *a*, using human urine.

ABNORMAL URINE.

1. Note change of smell, color, reaction, specific gravity, etc., in consequence of the standing of fresh urine for some time in a room at the ordinary temperature.

2. **Sediments.** *In acid urine* may be:

(a) Urates; dissolve on heating

(b) Uric acid in pepper-grain crystals; dissolve in strong alkali.

(c) Oxalates; dissolve in hydrochloric but not in acetic acid.

In alkaline urine, commonest sediment is phosphates, which dissolve on adding acetic or hydrochloric acid.

Other sediments (pus, blood corpuscles, etc.) are best recognzied by the microscope.

3. **Sugar** in urine can be detected by Trommer's test and the others mentioned in Chapter I, and by Fehling's solution (Chapter VIII).

VIII. Urinary Quantitative Estimation.

1. Quantitative Determination of Sugar.

Preparation of the Fehling's solution:

(*a*) Pure copper sulphate pulverized, and dried by pressing between folds of bibulous paper, 34·64 grams to be dissolved and diluted up to 500 c.c.

(*b*) Pure Rochelle salt (tartrate of sodium and potassium) in crystals, 173 grams to be dissolved in caustic soda (S. G. 1·34) 100 c.c. Dilute up to 500 c.c.

As both these solutions tend to change in composition, they must be kept excluded from air (stoppered bottle)

To make Fehling's solution add (*a*) to (*b*) gradually, and shake well if necessary to form a clear blue solution.

Keep excluded from the air.

10 c.c. of this solution reduces ·05 dextrose.

Method of determination of sugar:

(*a*) If the urine contains more than ·5 to 1 per cent. of sugar, it should be diluted.

(*b*) The apparatus required: A Mohr's burette with stand; a 10 c.c. pipette; a 200 c.c. flask; an iron tripod; a few very small flasks; funnels and filters.

(*c*) Place in a 200 c.c. flask 10 c.c. of the Fehling's solution and dilute with 40 c.c. of distilled water.

Fill the burette with the (diluted) saccharine urine.

Heat the flask till the contents begin to boil. Then, continuing the heat, let the urine flow from the burette cautiously. This is to be continued *till the blue color of the copper solution is wholly removed.*

To ascertain this: When the color is seen to be nearly gone, remove the flask from the heat, and after it has stood for a little hold it up before a window. If it be doubtful whether the fluid contents are colorless, filter a few drops through one of the filters into a small flask resting on white filter paper; if any color remains it can be discovered.

If the filtrate is colored, wash it back into the large flask and proceed with the titration.

Towards the end of the process the urine must be added a few drops at a time.

Three estimations should be made to insure a reliable result.

2. Estimation of Urea by the Hypobromide Method.

Preparation of the liquid:

Dissolve 100 grams caustic soda in 250 c.c. water and add 25 c.c. bromine. Preserve in a well-stoppered bottle.

$$2\,Na\,O\,H + Br_2 = Na\,Br + H_2\,O + Na\,Br\,O.$$

Methods of estimation with Dupré's apparatus;

Introduce into the mixing vessel 15–20 c.c. of the hypobromide solution.

Place in the receiver with a pipette 5 c.c. urine.

The measuring tube is to be placed in a cylinder filled with water at the temperature of the room.

Regulate the apparatus now so that there is no undue pressure, and see that the whole is air-tight.

Tilt the vessels containing the urine and hypobromide solution so that the fluids *gradually* mix ; when the effervescence has almost ceased, shake the containing vessel well and wait till all effervescence ends.

The measuring tube should be gradually raised as the liberation of gas proceeds.

When there is no more effervescence sink the measuring tube into the water of the cylinder.

See that no leaking of gases takes place. Place the mixing vessel in water of the same temperature as that in the cylinder for 5–10 minutes.

Read off the results after raising the measuring tube till the surfaces of the liquids inside and outside coincide.

37·3 c.c. of nitrogen at ordinary pressure and temperature correspond to 0·1 gram of urea.

Reactions :

$$CO\begin{cases} NH_2 \\ NH_2 \end{cases} + 3\,NaBrO + 2\,NaHO = 3\,NaBr + 3\,H_2O + Na_2CO_3 + N_2.$$

IX. Spectra of Blood.

1. **O-Hæmoglobin.** Dissolve a little blood clot in water; put from this into a test tube three parts filled with water, enough blood to show (*a*) but one narrow band; (*b*) two bands; (*c*) one broad band.

2. **Hæmoglobin.** (Reduced hæmoglobin).

(*a*) Add to a preparation corresponding to 1 *b* above, a few drops of $(NH_4)_2S$, and gently agitate.

One faint broad band only will be seen.

(*b*) Shake up or leave this exposed to the air for some time.

It will again show the bands of O hæmoglobin

3. **Methæmoglobin.** May be prepared by acting on Oxyhæmoglobin with an oxidizing agent, such as potassium ferricyanide. Brownish-red color. Gives a characteristic absorption band in the red between the *c* and *d* lines. If treated with a reducing agent yields hæmoglobin.

4. **CO-Hæmoglobin.** Allow illuminating gas (CO) to pass into defibrinated blood (or solution of hæmoglobin) for 20 minutes.

The blood becomes cherry red.

Examine with the spectroscope. Two bands will be seen in almost the same portion of the spectrum as those of O-hæmoglobin.

Attempt reduction as in (*a*). It is impossible

5. **Alkaline Hæmatin.**

Add to a solution of blood clot some solution of caustic alkali; warm gently. Note the change of color and the absorption band to the left of the D line.

6. **Reduced Alkaline Hæmatin** or hæmochromogen may be formed from alkaline hæmatin by the action of a reducing agent. It gives a very distinct band to right of D and a less distinct one near E.

X. BLOOD.

1. **Essentials for Coagulation.** Dilute some salted plasma with 10 volumes of water and divide into three portions in test tubes labelled *a*, *b*, *c*. Heat *a* to 60° C. To *a* and *b* add a drop of serum. Put all three tubes in test tube rack and examine in five minutes for clotting. If none has appeared in any of them examine again later. Think out explanation of results on the basis of the three essentials for clotting, viz: fibrinogen, fibrin-ferment, and a calcium salt. Divide oxalate plasma into two portions *d* and *e*. To *d* add a few drops of 2% solution of calcium chloride. Let tubes stand for half an hour and note results.

2. **Proteids of Serum.** Heat a portion of serum and note coagulation show. presence of globulins or albumins or both. Divide the remainder into two parts. To one part add an equal quantity of saturated solution of ammonium sulphate. Note the faint precipitate of serum globulin. Add an equal quantity of water to the other portion of serum and compare. Filter off the precipitate of serum globulin. Heat the filtrate, which is free from globulin, very gradually in a water bath with a thermometer in the tube. When a turbidity occurs remove tube from water bath, note temperature and filter. Heat filtrate till a fresh coagulum is observed and filter off as before. Repeat a third time. Coagulations are to be expected at 73° C., 77° C. and 84°C, corresponding to serum albumins *a*, *b*, *c*.

3. **Chemical Tests for Hæmoglobin.** Heat a solution of blood clot. Note change of colour to brown (Hæmatin). Add to some solution of blood clot a few drops each of tinct. of guaiac and H_2O_2. The blue colour which results is usually regarded as an indication of the presence of hæmoglobin although a number of other substances also give it.