PRACTICAL
PHYSIOLOGICAL CHEMISTRY

A BOOK DESIGNED FOR USE IN COURSES IN PRACTICAL PHYSIOLOGICAL CHEMISTRY IN SCHOOLS OF MEDICINE AND OF SCIENCE

BY

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PREFACE TO THE NI

It is with a feeling of distinct pleasu
before the friends of former editions of
Chemistry" with this volume which s
considerably above that of any previous

In addition to a most comprehensiv
entire volume, entailing the complete re
important chapters, it has been found n
upon the previous high standard of the
new chapters and sections. Therefore, n
of much subject matter from the previo
necessity been increased in size. It has b
life of this volume, to give to the stude
and the investigator the very best in t
conclusions, etc., which come to us as t
leading biochemists of the world. Becau
and widening scope of our science this ta
each revision.

It is not feasible to mention specificall
to make up the general excellence of th
mention of the new chapters and sectio
character of some of the more important

Seven new chapters have been includ
them covering subjects not heretofore c
new chapters are:

Chapter I:—"Physical Chemistry of
Chapter XIV:—"Absorption."
Chapter XV:—"Putrefaction and De
Chapter XVIII:—"The Chemistry of
Chapter XIX:—"Respiratory Meta
lation."
Chapter XX:—"The Endocrine Orga
Chapter XXI:—"Energy Metabolism

Chapter I includes understandable d
procedures of importance in biochemistry

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and mix. Note color and rapidity with which it develops. For tyrosine and tyramine make strongly alkaline with NaOH and then add a small amount of hydroxylamine hydrochloride solution. For details of separation and determination, see original papers.

2. Millon's Test.—Test the material with Millon's reagent. A red color is given by compounds containing the hydroxyphenyl group.

3. Bromine Test.—Add a few drops of bromine water. Cresols, phenol, and hydroxy aromatic acids give white precipitates of bromine derivatives.

PREPARATION OF PHENYLACETYLGLUTAMINE

$C_6H_5.CH_2.CONH.CH(COOH)CH_2.CH_2.CONH_2$

Although theoretically this compound could be made by a coupling reaction between phenylacetylchloride and glutamine, yet owing to the difficulty of obtaining the latter the compound is best obtained as the detoxication product of phenylacetic acid by the human organism.

Ingest 10 grams of phenylacetic acid in two 5 gram doses taken 24 hours apart, preferably after a meal. The substance is ingested as the water solution of its sodium salt, made faintly acid with hydrochloric acid. Collect the urine until 24 hours after the second dose and without adding any substance to it evaporate it on the water bath. Do not take to absolute dryness, but stop when the residue in the dish begins to froth. Make slightly acid to Congo red with 25 per cent sulphuric acid, and after thorough cooling add cautiously about 10 c.c. of concentrated sulphuric acid, mixing vigorously to avoid local overheating. Transfer the pasty mixture to a rotary extractor (Fig. 85) and extract from one to three hours with absolute ethyl acetate.[1] When the boiling ethyl acetate in the flask of the extraction apparatus begins to precipitate a brownish gum, disconnect the flask and quickly filter the liquid into a beaker immersed in ice water. Stir vigorously and let stand for some time in the cold. The mass of white crystals is then filtered off on a Buchner funnel and washed with cold ethyl acetate. The extraction may be repeated a number of times, each time taking out a little more of the desired substance, until so little crystallizes out that it does not pay to continue. The white crystalline precipitate is the molecular addition compound of urea with phenylacetylglutamine, mixed sometimes with free urea. The free urea and that combined with the substance are both removed together by means of urease; this serves to determine the amount of urea present, but is not satisfactory for the isolation of the pure substance.

Pure phenylacetylglutamine may be obtained from the urea addition compound as follows:[2] Dissolve the substance in water and make slightly alkaline with barium hydroxide. Do not warm the solutions. Remove the excess barium with carbon dioxide, filter, and evaporate at not too high a temperature on the water bath. The resulting mixture of urea and the barium salt of phenylacetylglutamine is dried thoroughly in a vacuum oven at about 60° and is then extracted three times with absolute alcohol, cooling after each extract, and filtering off the alcoholic solution of urea. The residue, i.e., the pure barium salt of

[1] The anhydrous ethyl acetate of the Eastman Kodak Co. or of the U. S. Industrial Chemical Co. is satisfactory for this purpose. Ordinary commercial ethyl acetate containing appreciable quantities of water and alcohol is useless.

[2] Sherwin, Wolf, and Wolf: *Jour. Biol. Chem.*, 37, 115, 1919.

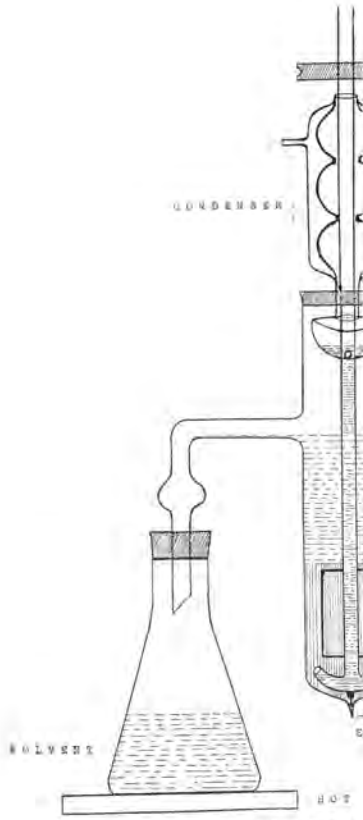

FIG. 85.—ROTARY EXTRA

[1] The condenser may be attached to the body of the e
or by a ground joint. The long glass tube running dow
tor opens out into a sort of curved T at the bottom and
fitting into the bottom of the extractor. Above this T
lar shape are sealed on to act as stirrers. Inside the
under the condenser a sort of glass cup is sealed on t
a small hole blown in it above its junction with the
hot-plate, and the vapor on condensing runs into the cu
the small hole. After a sufficient column of solvent
balance the pressure of the material being extracted
sistency) pure solvent will begin to flow out of the o
and will carry some dissolved substances up with it.
into the extractor proper will then overflow back into th
continually be getting richer in the substances being e
the apparatus prevents much of the "muck" in the b
into the flask. The stem carries a pulley at the top (a
will do) and by means of a suitable speed reduction dev
should make about 60–80 revolutions per minute.

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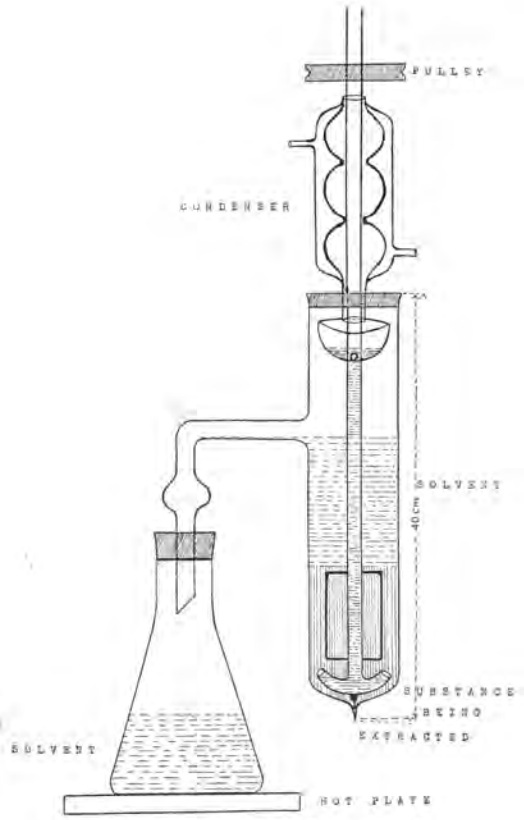

FIG. 85.—ROTARY EXTRACTOR.[1]

[1] The condenser may be attached to the body of the extractor by means of a cork stopper or by a ground joint. The long glass tube running down through the condenser and extractor opens out into a sort of curved T at the bottom and rotates on a platinum pin as bearing, fitting into the bottom of the extractor. Above this T, two glass rods bent into rectangular shape are sealed on to act as stirrers. Inside the body of the extractor and directly under the condenser a sort of glass cup is sealed on to the central glass stem, which has a small hole blown in it above its junction with the cup. The solvent is boiled on the hot-plate, and the vapor on condensing runs into the cup and thence down the stem through the small hole. After a sufficient column of solvent has backed up in the stem to overbalance the pressure of the material being extracted (which should be of a pasty consistency) pure solvent will begin to flow out of the openings at the bottom of the stem and will carry some dissolved substances up with it. The excess volume thus introduced into the extractor proper will then overflow back into the flask; so the liquid in the flask will continually be getting richer in the substances being extracted, while the general form of the apparatus prevents much of the "muck" in the bottom of the extractor from getting into the flask. The stem carries a pulley at the top (a flat cork stopper with a groove in it will do) and by means of a suitable speed reduction device is driven by a motor. The stem should make about 60–80 revolutions per minute.

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phenylacetylglutamine, is then dissolved in the smallest possible quantity of water, the calculated amount of phosphoric acid is added, and the barium phosphate filtered off. The aqueous solution is then concentrated at room temperature in vacuo and extracted with absolute ethyl acetate, yielding pure phenylacetylglutamine.

Estimation of Intestinal Reductions. Method of Bergeim.[1] **Principle.**—A definite amount of ferric oxide is mixed with the food. In the feces the percentage of the oxide which has been reduced is determined. This is an index of the intensity of reduction processes in the bowel.

Procedure.—Experiments may be made on albino rats. The animals under investigation are given any desired diet with which the iron salt used is incorporated to make a uniform mixture. A mixture with 1 per cent of hydrated ferric oxide powder (completely soluble in 100 parts of 12 per cent hydrochloric acid in about 30 seconds on the boiling water bath) or one-fourth this amount of a coarser ferric oxide (dissolving in about 2 minutes) may be used. The same preparation should be used throughout a given series of experiments.

The following diets may be tested:

(1) Starch 80, agar 1, ferric oxide 1, casein 20.
(2) Egg albumin instead of casein.
(3) No. 2 and 50 per cent of glucose.
(4) No. 2 and 50 per cent of lactose.
(5) Milk powder 98, agar 1, ferric oxide 1.
(6) Meat powder 98, agar 1, ferric oxide 1.

After 2 days on the experimental diet specimens of feces are collected. In the case of the rat these may be obtained by digital pressure on the lower bowel.

About 0.2 gm. of feces (not weighed) is rubbed up with 10 c.c. of dilute HCl (1:2) and the mixture heated in a test tube of about 18 mm. diameter on a boiling water bath for ½ minute (2 minutes if the coarser iron oxide is used). If biliary or other pigment be present in appreciable amount 0.5 gm. of acid-extracted blood charcoal should be added before heating. This is usually not necessary in studies on rats, but is required in the examination of human feces. The tubes are placed in cool water for about ½ minute and then filtered preferably within ½ hour. 2 or 3 c.c. aliquots of the filtrate are measured into each of two 25 c.c. graduated cylinders. To one add enough 0.1 N potassium permanganate solution to give a pink color lasting for about 1 minute (1 or 2 c.c. are usually required). This oxidizes the ferrous iron to the ferric condition. Then to each cylinder add 2 or 3 c.c. of a 1 per cent solution of potassium thiocyanate and make each to volume (usually 7 to 10 c.c.). Compare the two solutions obtained in a colorimeter.

Calculation.—Divide the lower by the higher reading, multiply by 100, and subtract from 100. This gives the percentage of ferric oxide reduced.

Interpretation.—Reduction is brought about primarily by bacterial action in the cecum and large intestine. Casein diets show much less reduction than those containing equal amounts of egg or meat protein. Lactose given in fairly large amounts, because of its slow digestion and absorption, reaches the lower bowel and is there fermented to produce lactic acid which inhibits the growth of putrefactive organisms and lessens reduction. Milk is therefore particularly effective in decreasing intestinal putrefaction and reductions.

[1] Bergeim: *Jour. Biol. Chem.*, 64, 45, 1924.

Reductions by Intestinal Bacteria. Test Tube
50 gm. of intestinal contents or feces with an eq
of 5 per cent dialyzed iron to which enough sodiu
added to neutralize the acidity. Strain through c
of the mixture into each of six test tubes.

To No. 1 and No. 2 add about 0.5 gm. powder
about 0.5 gm. of powdered calcium sulphate.
Heat Nos. 2, 4, and 6 in a boiling water bath f
Incubate all tubes over night at 40°. Note which
tion of the ferric hydroxide as indicated by darke

Introduce 2 c.c. of each mixture into a ser
aspiration. Add 1 c.c. of concentrated HCl to ea
test tubes each containing 5 c.c. of a solution of
per cent and lead acetate 0.05 per cent. A brov
sulphide is formed whose density is proportion
present. Is any of the sulphur or sulphate red
bacteria present? The bacterial processes occu
hours after passage are believed to be similar to t
After this the bacterial action may assume a diff

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