PRACTICAL METHODS IN PHYSIOLOGICAL CHEMISTRY.¹

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The increased attention that has been given in recent years to the study of auto-intoxication, and particularly the papers read at the meeting of this Society last year by Drs. Clark, Hill and Hurd, have emphasized the importance of a careful study of the secretions and excretions of the insane as stepping-stones to a rational theory of the nature and cause of certain forms of mental disorder.

The subject is one that concerns us very closely, for the opinion is gaining ground that a very large proportion of diseases of the nervous system, and particularly those symptoms of disordered mentality which we call insanity, are produced by irritation of the higher nervous centers by poisons introduced from without or elaborated within the body. One recent writer, Dr. V. C. Vaughan, says: "It is true, without exception so far as we know, that the excretions of all living things, plants and animals contain substances which are poisonous to the organisms which excrete them. A man may drink only chemically pure water, eat only that food which is free from all adulterations, and breathe only purest air, free from all organic matter, both living and dead, and yet that man's excretions would contain poisons. They originate in the metabolic changes by which the complex organic molecule is split up into simpler compounds. We may suppose—indeed, we have good reason for believing—that the proteid molecule has certain lines of cleavage along which it breaks when certain forces are applied, and that the resulting fragments have also lines of cleavage along which they

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break under certain influences, and so on until the end products—urea, ammonia, water, and carbon dioxide—are reached; also, that some of these intermediate products are highly poisonous, as has been abundantly demonstrated. The fact that the hydrocyanic acid molecule is a frequent constituent of the leucomains is one of great significance. It matters not whether the proteid molecule be broken up by organic ferments, bacteria, or by the unorganized ferments of the digestive juices, or by the cells of the liver, or by those still unknown agencies which induce metabolic changes in all the tissues, in all cases poisons may be formed."

Dr. Van Gieson, in speaking of the influence of auto-poisons in the production of nervous and mental disease, says: "It is my profound conviction that in the course of time the application of this toxic theory of disease to the nervous system is destined to clear away much of the present vagueness and mystery of the causative agents and pathogenesis of mental disease."

The opinion is general that to accomplish anything useful in chemical research long and special training is necessary; that one's entire time will be required to perform the work, and that the apparatus necessary is complicated and expensive. The experience that we have had at the St. Lawrence State Hospital does not uphold this view, for during the past year we have carried on almost constantly work in physiological chemistry in connection with the investigations being done at the State Hospitals' Pathological Institute in New York City, and we have found that we have been enabled to do this work with but little conflict with our regular duties, and it has seemed useful to record our experiences and to tell something of the equipment and work of our laboratory,³ even though in doing so there is nothing original that can be said. Our experience will be useful to any one contemplating inaugurating such work.

The methods which we follow are to be found in one or the other of the numerous text-books on physiological chemistry that can be had, but it would require considerable time to elim-

³ We take pleasure in recording our indebtedness to Dr. S. Bookman, Associate in Physiological Chemistry in the Pathological Institute, New York, for valuable assistance in the organization and equipment of our laboratory.

inate some of the methods that are not satisfactory, and to arrange a system that would give satisfactory results.

Our work has been done by the regular staff, and has not been permitted to interfere with the other duties of an assistant physician, and it seems that such work can be carried on in nearly any hospital.

The laboratory is in the executive building, convenient to the wards and adjoins the dispensary. If occasion requires, the apothecary can be called upon for assistance during a part of the day, and when the assistant physicians are engaged elsewhere, he can, by going in occasionally, see that the temperature of the oven remains uniform and that such processes as filtration, evaporation and digestion, which require considerable time, go on without accident. As a matter of fact, he is seldom needed with us, but in a hospital where the staff is small, the apothecary, familiar as he must be with many of the technical details of chemistry, may be made a useful ally.

The front of the room is occupied by a large window with a shelf in front for microscopical work and for titrating and delicate color tests of all kinds. Down the middle of the room runs a long work table 40 inches wide and 36 inches high, the top of which is slate, and it has a shelf beneath for bottles. The walls are shelved to a convenient height, and there is a cabinet for the more delicate apparatus and glassware. An indispensable feature of every laboratory of this kind is a ventilated cupboard, or a hood, to carry off irritating vapors. Our cupboard was made in a chimney; an arch was cut a little higher than the head and an ordinary window-sash fitted to the front balanced upon weights. Its floor is covered with galvanized iron and is about 36 inches above the floor of the room.

It is necessary for two or more members of the staff to work together. The several determinations can be arranged to begin in the morning at a convenient hour, say nine o'clock, when the time in the laboratory required will be about an hour; then little or nothing need be done until near noon, when half an hour's attention should be given the work, and again in the afternoon at two o'clock the entire work can be finished up within two hours. With the exception of the first part, the early morning hour, it would only be necessary for one man to be in the laboratory at

once, though of course some of the work can be done more rapidly by two than by one. The day may be divided up among the several men or they can work on alternate days.

When beginning the work of the day it is a good practice to get all the determinations started as early as possible; this particularly applies to those which require several hours for their completion, for while waiting for one solution to boil another can be filtered, and by working on each in turn all can progress through successive steps to completion without waste of time. To do this successfully, each flask and beaker must be labeled to show what its contents are and in what stage. This can be done quite simply; each complete specimen when received is given an accession number, for example 20, and an entry is made in the accession book opposite the number showing the date, name of the patient, the disease, and remarks of his condition during the day. This number is placed upon the jar containing the specimen and upon every dish or beaker into which any of it is measured. To this mark of identity is added another to show the determinations as "Cl" for chlorides, "Hu" for uric acid, and so on; if there are several stages, the final numbers 1, 2 or 3 show in which it is. A filter bearing the mark "20 S 2" would be known to contain the second or ethereal sulphates from specimen No. 20.

It is useless to undertake any quantitative determination on a specimen of urine of less than twenty-four hours, as its composition varies with sleep and waking and with digestion and fasting. The specimens, as often as passed, should be at once measured by the nurse, the reaction tested, the bottle labeled with the time, quantity and reaction, and brought to the laboratory to be put upon ice. The last specimen should be taken on rising in the morning and should be brought to the laboratory in time to begin work about nine o'clock.

The several specimens are then poured into a graduated jar and are well mixed. The reaction of the whole is taken and the quantity noted as a check on the separate measurements. From this time the mixed urine is looked upon as a single specimen, and it is particularly important to know the exact quantity to insure correctness in the several results. The specific gravity is tested by the urinometer, or, better, by the specific gravity

bottle. Five cc. are measured from a pipette into a platinum dish of known weight and put in the oven and evaporated to dryness for the total solids. After this the specimen is filtered, as the succeeding tests are best made with filtered urine.

The following are the methods we employ for the estimation of the constituents of urine:

CHLORIDES.—Place 10 cc. of filtered urine in a porcelain dish and add a few small crystals of sodium carbonate and sodium nitrate, after which it is evaporated to dryness over a water-bath. The dish is then heated gradually over a Bunsen flame until the residue ignites and burns away. The heat is continued until the residue assumes a whitish color, but the temperature should not be sufficiently high to volatilize the salts. After the dish has been cooled, 20 cc. of water are added, together with a few drops of nitric acid, which is afterward neutralized by calcium carbonate. The solution is then boiled, filtered and washed. To the clear filtrate a drop or two of solution of potassium chromate are added for an indicator and titrated with a 2 per cent. solution of nitrate of silver. This is done by adding to the filtrate the nitrate of silver solution from a graduated burette, drop by drop, with constant stirring, until a reddish tinge occurs which does not disappear. This indicates that the process is completed. Now read off from the burette the number of cc. of silver solution that have been used and multiply the result by 6.88, which gives the number of milligrammes of sodium chloride in the 10 cc. of urine employed.

URIC ACID.—Place 50 cc. of filtered urine in a beaker and add 5 cc. of ammonio-magnesium solution.* It is then heated over a water-bath for about half an hour and titrated with a N/50 solution of nitrate of silver, using a drop of ammonium sulphide as an indicator. This titration requires considerable care, as the process must be completed before the urine is cooled and after adding the nitrate of silver. The indicator cannot be applied until the urine has been filtered. This obstacle is overcome by

**Ammonio-magnesium solution:*

20 grams magnesium sulphate,
30 grams ammonium chloride,
125 cc. ammonium hydrate,
125 cc. distilled water.

having a very small filter, which will hold five or six drops. One cc. of the silver solution is added to the urine, and, after stirring, a few drops of the solution are removed in a pipette and filtered through the small filter and a drop of the clear filtrate permitted to fall upon a drop of ammonium sulphide upon a white surface. If the ammonium sulphide is colored dark, it shows that too much of the solution has been added, but if no result is obtained, the process is continued cautiously, adding $\frac{1}{2}$ cc. of silver solution at a time until a faint color is produced in the indicator. The number of cc. of silver solution employed is then read off from the burette, $\frac{1}{2}$ cc. is deducted to correct a slight error, and the number multiplied by 3.36, the product of which represents the number of milligrammes of uric acid in the urine employed.

PHOSPHATES.—Take 50 cc. of filtered urine and add 5 cc. of a standard solution of sodium acetate. It is then heated on the water-bath about half an hour and titrated while hot with normal solution of uranium nitrate until a drop of the mixture gives a brown color with a drop of potassium ferrocyanide on a porcelain slab. Multiply the number of cc. of solution used by .005, which gives the amount of phosphoric acid in 50 cc. of urine.

SULPHATES.—Measure 50 cc. of filtered urine into a beaker and add 10 cc. of 30 per cent. glacial acetic acid. This is then placed upon the water-bath for twenty minutes and 15 cc. of hot saturated solution of barium chloride are added with constant stirring. The heat is continued for a few minutes and the beaker is then put aside in the ice-box until cold. It is then filtered through an ashless filter and well washed with hot distilled water and dilute HCl until a drop of the filtrate gives no cloudiness upon addition of diluted sulphuric acid. The filtrate and washings are put aside and the filter containing the precipitate is removed carefully with a metal spatula and placed in a platinum crucible of known weight and heated over a Bunsen flame until the ash is white. After cooling, a few drops of nitric acid and of sulphuric acid are added and the crucible is again heated, at first cautiously until the fumes disappear, and then to redness, after which it is cooled in a desiccator and weighed. The increase of weight represents the preformed sulphates in the form of sulphate of barium. The filtrate and washings which were put aside are mixed with 15 cc. concentrated hydrochloric acid

and boiled for five minutes and put in the ice-box to cool, then filtered through an ashless filter and burned in a platinum crucible and weighed as described for the preformed sulphates.

TOTAL SOLIDS.—5 cc. of unfiltered urine are carefully measured into a platinum dish of known weight and placed in the oven at a temperature of 95 to 100 degrees for five hours, then removed, cooled in the desiccator and weighed. The weight, after deducting the weight of the dish, represents the total solids in 5 cc. of urine employed. The dish is then heated to dull redness over the flame until all moisture is expelled; when cool, a few drops of nitric acid are added and it is again heated until the residue is white. Finally a few drops of sulphuric acid are added, and after heating to redness again it is cooled in a desiccator and weighed. The increase of weight after subtracting the weight of the dish represents the inorganic solids as sulphates.

AMMONIA.—20 cc. of filtered urine are measured into a shallow dish and mixed with 10 cc. milk of lime and placed under a bell jar with 10 cc. deci-normal solution of sulphuric acid in another dish. Everything should be made ready and the two dishes instantly covered by the bell jar as soon as the lime is added, or some of the ammonia will be lost. It is left undisturbed for 48 hours, when the acid is titrated with deci-normal solution of sodium hydrate and the diminished acidity is evidence of the quantity of ammonia absorbed.

UREA.—For the estimation of urea, we employ the method of Prof. Hüfner, which consists in decomposing the urea into water, carbonic acid and nitrogen by means of an alkaline solution of hypobromite of soda. The water and carbonic acid are dissolved in the solution, and the nitrogen which collects in a graduated tube is measured and from it the urea is estimated. 35.4 cc. of nitrogen represents 0.1 gramme of urea.

If the urine contains albumen it must be acidified, boiled and filtered before applying this test.

SODA AND POTASH.—The salts of potassium and sodium can be conveniently estimated together by the following method: To 50 cc. of urine are added an equal volume of saturated solution of barium hydrate; it is allowed to stand for a short time and is filtered. 50 cc. of the filtrate are collected and evaporated to dryness in a porcelain dish over the water-bath and the residue

gently heated over the flame until the organic matter is destroyed. When cold, dissolve in hot water and add ammonia carbonate as long as a precipitate occurs, then filter and wash. The filtrate is acidified with hydrochloric acid and evaporated to dryness in a weighed crucible on the water-bath and then in the flame until white fumes cease. The weight, minus the weight of the crucible, is that of the sodium and potassium in the form of chlorides. To separate the two salts, they are dissolved in a little water and platinic chloride added to excess and the mixture evaporated to half its volume. A few cc. of alcohol are added, which dissolves only the sodium salt, the potassium salt remaining unaffected. They are separated by filtration and washing with alcohol; the insoluble potassium salt which remains in the filter is weighed after drying carefully, and its weight deducted from the weight of the combined salts gives the weight of the soda. 100 mg. of potassic chloride of platinum is equivalent to 30.51 mg. of chloride of potassium.

TOTAL NITROGEN.—5 cc. of urine are mixed in a Kjeldahl flask with 15 cc. of pure concentrated sulphuric acid and digested over a low flame until the fumes cease to come off and the liquid assumes a straw color. A few small crystals of permanganate of potassium are then added to complete oxidation, and after five minutes further heating the flask is removed and cooled. It is then diluted cautiously with water, mixed in a distilling flask with an excess of caustic soda solution, and distilled into a known quantity of deci-normal solution of sulphuric acid. When the mixture in the distilling flask has been reduced to half its volume the process is completed and the quantity of nitrogen (in the form of ammonia) determined by titrating the deci-normal acid with a corresponding solution of soda.

TOXICITY.—The toxicity of urine is best tested by its poisonous effect when injected into the circulation of animals—white rabbits are probably best for this purpose. Young adults should be selected, free from injury and of lively disposition. The following observations are noted before beginning the experiment, viz. weight, temperature, pulse, respiration and the size of the pupils.

The syringe we use was made for the purpose and can be had in the market. The barrel is of glass, 50 cc. capacity and

graduated to 5 cc.; the needle, the size of an ordinary hypodermic needle, is connected to the barrel by a short piece of india-rubber tubing.

The rabbit is fastened to the table or a board in such a way that it cannot struggle violently and the needle inserted in one of the superficial veins, being careful that no air is in the syringe or tube. The marginal vein of the ear is usually selected. It can be seen in rabbits with white ears by holding the ear to the light. In rabbits with black ears, it is sometimes difficult to find. It runs one-eighth of an inch from the border. Having made sure that the needle is well in the vein, the exact time is noted and the injection begun. The rate of injection should be uniform and about 5 cc. per minute. As the experiment proceeds, notes should be made of the symptoms shown, noting the time and the amount of the urine injected. The commonest symptoms observed are changes in the frequency and rhythm of the respirations, contraction or dilation of the pupils, spasms of groups of muscles and general convulsions, which frequently end in death. It is better to continue the injection until death occurs, as no accurate data are obtained if the animal survives.

The toxicity of urine varies considerably in different individuals and in the same individual with variations of diet and exercise, and no attempt to establish a standard need be made without taking these factors into consideration. According to Bouchard, who is perhaps our best authority, 30-60 cc. of normal urine will cause the death of a rabbit weighing one kilo, and the same author estimates that the average human being would survive about 52 hours if the renal functions were entirely suspended.

The nature of this auto-poison has been long in doubt. It has been thought at different times to be urea, uric acid, ammonia and the potassium salts. Schiffer has shown that urine is still toxic, though in less degree, when the inorganic salts have been removed, and Lepine seems to have arrived very near the truth when he states that 80 to 85 per cent. of the toxicity of normal urine is due to inorganic salts and 15 to 20 per cent. to organic constituents. No reliable data have yet been obtained regarding the toxicity of urine in disease.

TOXICITY OF BLOOD.—The blood is obtained from the patient's arm with the usual antiseptic precautions, and is collected

in a sterilized flask; when sufficient has been obtained it is put aside in the refrigerator for 24 or 30 hours until a firm clot forms and settles to the bottom. The clear serum is poured off and filtered through a Berkfeldt filter; the following tests can then be applied in the same manner as for the urine. Specific gravity, urea, uric acid, chlorides and total nitrogen, and the toxicity determined by injection into the vein of a rabbit. The toxicity of the blood is usually found to be greater than of the urine and a smaller quantity will be required for the experiment, but it is well to select for the purpose a rabbit of not more than two pounds weight.

This important subject is still but little understood; there yet remains the important task of distinguishing auto-toxic disease when it is met with at the bedside, of tracing to its source the materies morbi, whether it originates in the intestinal canal or in some gland whose secretion is vitiated or suppressed, or whether in the nervous structures themselves, and suggesting methods of combating its deleterious effects. The solution of these problems can only come when the observations of many investigators can be collected and analyzed. It is safe to say that every hospital contains patients whose insanity is considered to belong to this category, yet reports of such cases are seldom seen in current literature. I would urge upon those who are establishing pathological laboratories to provide also an equipment for the accurate study of the blood and other fluids during life, feeling sure that in no other way can an intelligent construction be placed upon the delicate changes in nervous structure which are frequently revealed by the microscope.