Roentgenogram of arm.

we were almost ready to believe him, and were trying to figure out how a man of his age could be possessed of a cyst under his arm; and if a cyst, from what did it originate? However, on closer examination the tumor was found not to be perfectly oval in shape, but to be somewhat lobulated. It did not seem to be adherent to the deeper structures below, and it was not adherent to the skin above, although when the tumor was moved the skin above it would become somewhat wrinkled. It was quite freely movable and could be moved about on his arm quite easily. It was not in the least infiltrated

Patient, showing tumor of right arm.

and the skin at its point of attachment could be almost approximated with that on the other side. To be absolutely sure, however, that it was not a malignant affair, an x-ray was taken of the arm to rule out the possibility of any involvement of the bone, which might have been excited by the old injury. We were fairly certain, however, that it could not be sarcomatous or carcinomatous, because of the consistency of the mass, and the fact that the patient was in such good general condition.

Having satisfied ourselves that this was benign, the differential diagnosis must rest between one of three things:

1. Lipoma.

In favor of this we have a large, oval, rather lobulated, semi-fluctuating mass, which grew very insidiously and has never caused the patient any pain, or even discomfort outside of the fact that being so large it became a mechanical hindrance to him. It was subcutaneous and not fastened tightly to the skin or to the deeper tissues beneath.

2. Fibroma of the soft type. (Fibroma Molle.)

There was nothing which could really enable us to rule this out—although if it were a fibroma we would be inclined to expect it to appear more suddenly than this tumor mass did. Also the fact that it was somewhat lobulated would be more in favor of a lipoma, but would not by any means rule out a fibroma.

3. Myxoma.

These generally occur as rounded tumor masses, and from the characteristics of the tumor itself I do not think it could be differentiated from either a fibroma or a lipoma. These tumors sometimes originate in the sheath of nerve trunks, and if this was the case this would be an excellent location to expect such a growth. All of these conditions were considered and before operation opinion was still divided as to whether it was a lipoma or a fibroma.

At operation the mass was easily enucleated by Dr. Curran, and the tumor shown in the accompanying photograph was removed. It was found to weigh 2.5 kilograms, and measured 28x19x11 centimeters. The pathological examination showed it to be a typical lipoma.

The patient's arm has rapidly healed up and he is now very much elated that he is free from this cumbersome mass.

A NEW METHOD FOR THE ESTIMATION OF PEPTIC ACTIVITY OF GASTRIC CONTENTS, SUITABLE TO CLINICAL APPLICATION.

BY C. W. MCCLURE, M.D., BOSTON, AND
P. L. E. SCHABACKER, BOSTON.

[From the Evans Memorial.]

THE present communication is the description of a practical method for the estimation of pep-

tic activity of gastric contents, conforming to the general principles of enzyme action as at present understood; and of the application of the method to the determination of peptic activity of the stomach contents of human beings.

Up to the present the determination of relative quantities (concentration) of pepsin in stomach contents, as ascertained by the estimation of peptic activity, has been of very little assistance in explaining gastrointestinal symptomatology. One of the several reasons for this lies in the fact that methods for estimating peptic activity in gastric contents have given such variable results that only most inaccurate knowledge of the concentration of pepsin present could be obtained. The variability in results is due to the fact that the methods have not conformed to the general principles resulting from the application of physical chemical concepts to enzyme action. Obviously, a method conforming to these principles is necessarily one of the first steps in determining whether possible abnormalities in peptic activity play a rôle in the production of gastrointestinal symptomatology, and this is the reason for the present study.

The reagents required and the technic of procedure of the new method are as follows:

Great care was exercised in the preparation of the buffer solution, as it was felt that the uniformity and purity of this factor determine in large measure the course of all enzymic digestions.

A stock solution of hydrochloric acid was prepared by distilling a 20 per cent. pure acid in quartz, collecting the middle third of the middle third, and diluting the distillate to the desired strength (about half normal) with freshly distilled carbon dioxide free water. The strength of the acid was determined by exact gravimetric analysis as silver chloride.

Sodium hydroxide was prepared free from carbonate in a manner recently described,¹ and which may be summarized as follows: Forty-five grams of initially very poor sodium hydroxide were dissolved in 500 c.c. of freshly distilled water, the carbonate content determined by titration at 0° C., precipitated as carbonate with an excess of pure barium hydroxide, and the excess of the latter, after careful gravimetric analysis, removed as the sulphate with the calculated amount of pure sodium sulphate. The strength of the alkali was determined by titrating with the acid as described above, using weight-burettes and methyl red as the indicator. It was stored in paraffin-lined bottles and protected against carbon dioxide contamination by a soda lime tower.

Crystallized citric acid of the highest purity was next dissolved in carbon dioxide free water, the resulting solution being slightly in excess of half molal. Exact analyses of its strength were made with the sodium hydroxide described above, again using a weight-burette for the lat-

ter. Into a one-liter graduated flask was measured exactly enough of the citric acid to give 52.52 grams of the original solid. To this was added by weight enough of the sodium hydroxide to produce two-thirds neutralization of the acid. The volume was then made up to one liter, giving a fourth molal solution. This was used as the stock solution, keeping it in a paraffin-lined bottle and diluting with carbon dioxide free water to the final concentration of M/16 disodium monohydrogen citrate. To all solutions containing the sodium citrate thymol crystals were added to prevent the growth of molds.

N/10 HCl is made by diluting the acid described in the foregoing section to proper concentration, and titrating the strength with the standard NaOH also described, using weighing burettes and methyl red as the indicator.

The sodium citrate-hydrochloric acid mixture is prepared by pipetting 300 c.c. of the M/16 sodium citrate solution into a liter volumetric flask, rinsed with N/O HCl, and making up to the mark with N/10 HCl.

Edestin solution: One (1) gram of edestin,* weighed within an accuracy of 1 per cent., is placed in a mortar. The edestin powder is then titrated with a few c.c. of the sodium citrate-hydrochloric acid mixture to make a paste; by rapid trituration and slow addition of the sodium citrate-hydrochloric acid mixture the edestin powder can be kept in a finely divided state, which will allow it to go into solution readily. A total volume of 100 c.c. of the acid mixture is added from an ordinary graduated measuring cylinder. A small amount of residue will not dissolve, and is readily gotten rid of by centrifugalization and decantation of the supernatant fluid.

Twenty-five per cent. metaphosphoric acid solution: The preparations of metaphosphoric acid usually obtained in the market should be fused in a graphite mortar until the white fumes of phosphorus pentoxid begin to appear. The fused material can be cooled conveniently by pouring on a clean pie-pan floating on cold water. Metaphosphoric acid so fused will remain a potent protein precipitant if kept in tightly stoppered bottles.

The 25 per cent. solution can be made quickly by triturating the desired amount of metaphosphoric acid and water in a mortar.

Digestion mixture and Nessler's solution are prepared as directed by Folin and Wu,² the standard ammonium sulphate solution as directed by Folin and Denis.³

TECHNIC.

Centrifugalize or filter a small portion of the gastric contents and place one (1) c.c. of the clear fluid obtained in a 100 c.c. volumetric flask, then fill up to the mark with the sodium citrate-hydrochloric acid mixture.

*The edestin preparation was furnished through the courtesy of the Arlington Chemical Company, Yonkers, N. Y. The authors take pleasure in acknowledging indebtedness for their cooperation.

Pipette 9 c.c. of the 1 per cent. edestin solution into test tubes (100 mm. x 10 mm.) and place in a water bath at 40° C. for five minutes. Then add one (1) c.c. of the diluted gastric contents to each tube, shake and replace in the water bath for a period of 60 minutes. At the end of this time remove the tubes and immediately add 2 c.c. of the 25 per cent. metaphosphoric acid solution, shake violently, allow to stand 60 seconds and filter, using dry filter paper. The filtrate should be perfectly clear. One (1) c.c. of the filtrate is Kjeldahled according to the micromethod of Folin and Wu,² in the following manner: To each of two of the large Pyrex tubes, recommended by Folin and Wu, one (1) c.c. of the filtrate is added. Then to one of the tubes one (1) c.c. of the sulphuric-phosphoric acid mixture is added and 2 c.c. to the other tube. Digestion is carried out as described by Folin and Wu.² In brief,⁴ this consists in boiling off the water and, when the white fumes of sulphuric acid begin to develop, the mouth of the tube is covered by inverting a small Erlenmeyer flask into its mouth. Digestion is continued for from 30 to 60 seconds after the last trace of brown color has disappeared. The tube is then allowed to cool for 60 to 70 seconds, from 5 to 10 c.c. of water are quickly added and the contents of the tube cooled. The contents of the tube, to which the 2 c.c. of the acid mixture were added, are transferred quantitatively to a 100 c.c. volumetric flask and the total volume made up to about 60 c.c. with water. Then 30 c.c. of Nessler's solution are added and the flask filled up to the mark with water. The nesslerized solution is centrifugalized to get rid of the sediment* and compared with standard ammonium sulphate solutions usually containing 0.25 and 0.5 mgm. nitrogen. These are prepared by placing 2 c.c. of the acid mixture in a 100 c.c. volumetric flask, adding 5 or 10 c.c. of standard ammonium sulphate solution containing 0.05 mgm. per c.c., about 60 c.c. of water, 30 c.c. of Nessler's solution and making up to the mark with water. If sufficient color does not develop in the flask containing the Kjeldahled filtrate, then the other tube is to be nesslerized. To this tube add water up to the 35 c.c. mark, 15 c.c. of Nessler's solution, and, if necessary, centrifugalize to get rid of any sediment. While this procedure practically doubles the work of experiment, it gives an elasticity to the method which experience has shown to be essential.

The total number of milligrams of nitrogen found in the filtrate, after the metaphosphoric acid precipitation, is taken as an index of peptic activity. Using this index, the peptic activity of gastric contents obtained from a small number of normal young men, has varied between 3.6 mgms. and 5 mgms. These gastric contents

*The sediment must be pure white and not discolored by the precipitation of any of the coloring matter.

were obtained an hour after the ingestion of the Ewald test-breakfast.

DISCUSSION.

Methods representative of those devised in the past for the estimation of the peptic activity of gastric contents may be divided into the following classes:

1. Those in which gastric contents act on a substratum in the form of a solid mass placed in them or in a dilute HCl solution to which gastric contents are added: To this class belong the methods of Mett and Grützner. The Mett method⁵ is the well-known one of measuring the lengths of coagulated egg albumin in capillary glass tubes, before and after placing them in a solution containing pepsin. In the method of Grützner⁶ pepsin is allowed to act on fibrin which has been infiltrated with carmin. The amount of carmin liberated is determined colorimetrically and is used as an index to peptic activity.

2. Those in which gastric contents act on a flocculent suspension of edestin, in dilute solution of HCl: The complete disappearance of the precipitate is taken as the end-point of digestion. To this class belong the methods of Farrington, Lewis and Brown,⁹ and of Brewster.⁷

3. Those in which gastric contents act on some protein dissolved in dilute HCl: In the method of Fuld,⁸ edestin is dissolved in diluted HCl, and mixed with an equal volume of gastric contents. The end-point of digestion is considered to be reached when ammonia, layered on top of the mixture, no longer gives a white ring. Gastric contents are added to the solution of an acid casein dissolved in dilute HCl, in the methods of Volhard⁸ and Gross.⁸ In the latter method the end-point of digestion is reached when no cloudiness is developed upon the addition of sodium acetate. In the method of Volhard the mixture of gastric contents and casein solution are incubated a specified length of time. Then the undigested casein is precipitated by the addition of sodium sulphate and filtered off. The filtrate is titrated with N/10 NaOH, against phenolphthalein, and the increased acidity is used to express the amount of peptic action which has occurred.

Schorer⁵ has developed a refractometric method for the estimation of the action of pepsin, and Northrup⁸ an electrical conductivity method. These methods are not practical for clinical use and will not be discussed.

In criticising such methods as those outlined, or in devising new methods which are clinically applicable to the estimation of enzymatic activities of fluids obtained from the gastrointestinal tract, it is important that certain factors be considered. These factors⁴ are (1) quantitative accuracy and practicability of procedures for estimating the amount of enzyme action; (2) uniformity of enzymatic activity; (3) proportionality of enzyme action; (4) stability of the

enzyme, and (5) the physical state of the medium in which the enzyme is to act.

Uniformity of enzyme action means that under a certain set of experimental conditions a given specimen of gastric contents will give the same results on repeated analyses. By proportionality is meant the relation existing between the amounts of material digested by varying quantities of gastric contents; *i.e.*, in the method here described 2 c.c. of the diluted gastric contents will digest one and one-half times as much edestin as will 1 c.c. of the same dilution. Stability of an enzyme is a term used to express the length of time an enzyme will remain active. The physical conditions under which an enzyme acts are important because in order to obtain uniformity of action the physical conditions must be constant. One essential reason for this is the fact that the size of the surface area of material exposed to enzyme action is a governing factor in the amount of the latter which will occur. Other factors are temperature, and the buffer conditions determining hydrogen ion concentration.⁹

In classes 1 and 2, under description of previous methods, the solid state of the protein to be acted upon allows for great variation in the surface areas of different preparations. In none of the three classes of methods was an attempt made to find hydrogen ion concentrations and buffer conditions which would bring about uniformity of enzyme action, nor was considered the effect of adding the gastric contents on the pH and buffer conditions of the medium in which the pepsin was to act. Furthermore, in most of the methods the technic used does not permit an accurate estimation of the end-point of digestion. From these remarks it is obvious that methods previously proposed for the estimation of peptic activity of gastric contents have not fulfilled the five conditions described above, observance of all of which are considered essential for a method whose use is designed to give information concerning relative quantities (concentration) of pepsin present in different specimens of gastric contents.

While devising the new method here described an attempt was made to find pH and buffer conditions which would give uniformity of peptic activity of gastric contents. During this attempt results were obtained which indicated that a sodium citrate-hydrochloric acid mixture modified the action of pepsin. Further investigation led to the mixture described under the technic of the method; and under the experimental conditions outlined uniformity of peptic activity is obtained. The proportionality obtained is 1:1.5; *i.e.*, 2 c.c. of diluted stomach contents will digest one and one-half times as much edestin as will 1 c.c. of the same dilution. Under the experimental conditions outlined it has been found that pepsin remains stable for a considerable number of hours. The physical condition of the edestin is that of a solution, so that the

factor of surface area is constant; and the possibility of disturbances in hydrogen ion and buffer conditions on the addition of the gastric contents has been obviated by sufficiently diluting the latter. This brief discussion shows that the new method is in harmony with the factors generally accepted as determining enzyme action, and which are considered essential for the estimation of enzymatic activity. Further, it is directly applicable to determining this factor in the gastric secretion, and permitting the possibility of an interpretation of results for physiologic or pathologic purposes. The procedure for digesting the filtrate, given in the description of the technic of the method, allows the determination of widely varying degrees of peptic activity. This makes easier the application of the method to the study of pathologic gastric physiology.

483 Beacon Street.

REFERENCES.

- 1 Richards, and Rowe, A. W.: Jour. Am. Chem. Soc., 1922, xlv, 689.
- 2 Folin, O., and Wu, H.: Jour. Biol. Chem., 1919, xxxviii, 81.
- 3 Folin, O., and Denis, W.: Jour. Biol. Chem., 1916, xxvi, 491.
- 4 McClure, C. W., Wetmore, A. S., and Reynolds, L.: Arch. Int. Med., 1921, xxvii, 706.
- 5 Sahli, H.: Lehrbuch der klinischen Untersuchungs-Methoden Leip. und Wien, 1920, i, 614.
- 6 Farrington, P. R., Lewis, R. C., and Brown, P. W.: Jour. Lab. and Clin. Med., 1918-19, iv, 635.
- 7 Brewster, J. F.: Jour. Biol. Chem., 1921, xlv, 119.
- 8 Northrup, J. H.: Jour. Gen. Physiol., 1919-20, ii, 113.
- 9 Sørensen, S. P. L.: Biochem. Ztschr., 1909, xxi, 301.

FRACTURE-DISLOCATIONS OF THE HUMERAL HEAD.

BY WELLER VAN HOOK, A.B., M.D., CHICAGO.

It is time for surgeons to recognize that a revolution has taken place in the open treatment of joint-injuries and diseases through the knowledge that subcutaneous fat-tissue can be excised from its seat, even without pedicles, and replanted successfully in injured joint-cavities. For this artifice makes it possible safely to leave in the joint-cavity bone surfaces not covered by periosteum or cartilage, and synovial membrane but covered by fat-tissue. Even defects of joint-capsules and ligaments result in minimal disturbance of function if the space is filled in with fatty connective tissue.

The mode of action, the meaning of this effect of fat transplantation needs a moment's thought for its full comprehension. Fat-tissue is areolar connective-tissue having a tendency to the storing of oil in the cell-bodies. This tissue has an abundance of lymphatic spaces and channels and a fair degree of vascularity. It is probably not exceeded in adaptability by any other tissue in the body. Everyone knows how easily the areolar tissue retracts when illness occurs, how the eye-ball stands out, the cheeks shrink, and wrinkles appear, and how, in certain diseases, such changes as those just mentioned become relatively permanent. A laborer, exhausted by a day's activity, shows