30 to 50 minutes after glucose ingestion than it was after 60 to 80 minutes. The former time corresponds closely with the occurrence of the maximum level of glucose in the blood in glucose tolerance tests on normal individuals. Unfortunately it was impossible for us to measure blood glucose or R.Q.

Conclusions. 1. The preflight administration of a single dose of glucose in water to individuals on a normal diet produced a significant increase in the resistance to unconsciousness from anoxia at 27,000 and 30,000 ft. 2. The protection afforded flying personnel by the glucose solution was greater 30 to 50 minutes following its administration than after an interval of 60 to 80 minutes. 3. At 27,000 ft. the mean duration of consciousness was increased by approximately 40%, or more than one minute, by the preflight ingestion of glucose solution. 4. Vitamin C, either alone or in conjunction with glucose, had no demonstrable effect on the duration of consciousness.

15567

Total Peptic Activity of Gastric Juice. A Method of Determination.*

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It is the purpose of this paper to assess critically the present status of tests for the determination of pepsin and to describe an improved method.

Since different proteins differ in their rate of digestibility, it is necessary to choose either a highly purified protein for use as a substrate, or at least a substance of constant composition so that results be reproducible with different lots of substrate. Partial denaturization of a protein by coagulation renders it a mixture of different proteins. Because one can rarely be sure denaturization is complete, tests using coagulated protein substrates[1-3] are subject to error. Gelatin has been employed as a substrate protein,[4,5] but it is not ideal since it hydrolyzes in acids without pepsin.[6] One test uses fresh milk as a substrate.[7] Although the composition of "Klim," dried milk powder, is quite constant, individual samples of fresh milk are not constant.[8]

Pepsin is unlike many inorganic catalysts whose presence in traces is sufficient to bring about a change. With pepsin, the rate of conversion is directly proportional to the concentration of active enzyme and active substrate; *i.e.*, the reaction follows the law of mass action. However, the reaction rate is not uniform for more than a brief period (Fig. 1) because peptones formed during digestion combine with the enzyme to form an inactive pepsin-complex.[9] Pepsin + Peptone $\rightleftarrows$ pepsin-peptone. It is the concentration of peptone that determines how much pepsin is active. When the reaction rate has leveled off, the addition of a sizable increment of pepsin will but slightly increase the rate of digestion because of the high concentration of peptones already present. Dilution by decreasing the concentration of the inhibitor allows digestion to continue.[9] The

* Done on a grant from the Otho S. A. Sprague Memorial Institute.

1 Mett—Hawk and Bergein, *Practical Physiological Chemistry*, 11th Ed., 1937, p. 308.

2 Neilson, C. H., and Bonnot, E., *Arch. Int. Med.*, 1913, **11**, 395.

3 Riggs, B. C., and Stadie, W. C., *J. Biol. Chem.*, 1943, **150**, 463.

4 Osterberg, A. E., Vanzant, F. R., and Alvarez, W. C., *J. Clin. Invest.*, 1933, **12**, 551.

5 Gilman, A., and Cowgill, G. R., *J. Biol. Chem.*, 1930, **88**, 743.

6 Northrop, J. H., *J. Gen. Physiol.*, 1921, **3**, 715.

7 Barowsky, H., Tauber, H., and Kleiner, I. S., *Am. J. Digest. Dis.*, 1937, **4**, 229.

8 Northrop, J. H., *J. Gen. Physiol.*, 1932, **16**, 41.

9 Northrop, J. H., *J. Gen. Physiol.*, 1920, **2**, 471.

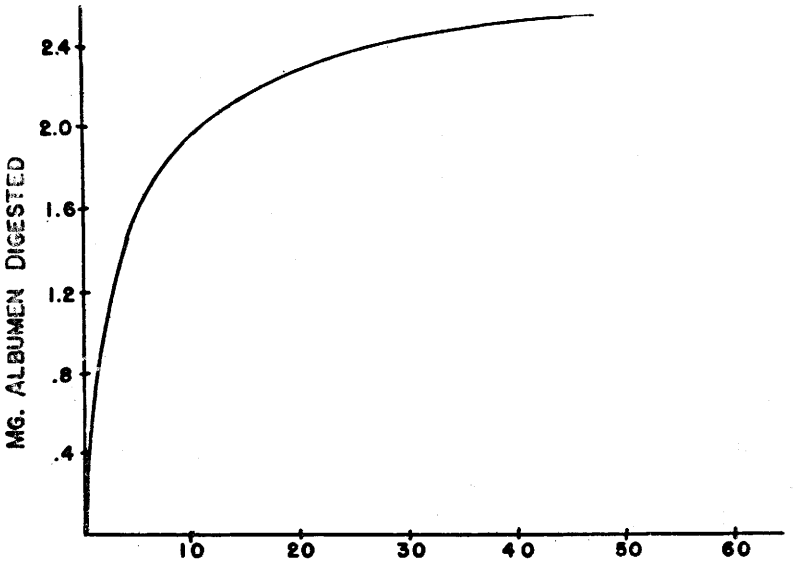

FIG. 1.
Milligrams of protein digested plotted against time.

pepsin of gastric juice is in combination with inhibitory substances which are probably peptones. It is rapidly and completely freed from the inhibitors by dilution. Some authors have found the separation usually to be complete at a 1/100 dilution.[10] It follows then that total pepsin can only be assayed on highly diluted gastric juice, since in low dilutions one is assaying not total pepsin but a varying mixture of pepsin and pepsin-inhibitor complex. One should therefore avoid tests using fixed low dilutions[2,3,11-13] and varying dilutions as an integral part of the test.[7,14]

A linear relationship does not exist between pepsin concentration and quantity of protein digested.[9] One should be able to express results in amount of protein digested (since it is a measure of peptic power) or in some figure proportional to the pepsin concentration. The liberation of tyrosine during digestion of a hemoglobin substrate occurs almost in direct proportion to enzyme concentration.[15] In the test to be described, the mg albumen digested are proportional to the logarithm of the pepsin concentration. In most tests, the kinetics of the reaction have not been investigated.

Although most methods for determining pepsin fail to meet all the criticisms, one modification of Anson and Mirsky's method is satisfactory.[10] This new modification, as stated by the authors and by the experience of others, requires considerable technical skill and is somewhat time-consuming. These factors render its use of limited value in most clinical and investigative work. For this reason, the method of Mett is still extensively used although its extremely wide range of error has been known for some time.[16]

Previous tests have one great disadvantage: there is no scheme for assaying total pepsin and the percentage of pepsin inhibited in gastric juice.

Dennis and Ayer[17] have described an accurate method for the determination of pro-

[10] Bucher, G. R., Grossman, M. I., and Ivy, A. C., *Gastroenterology*, 1945, **5**, 501.

[11] Waldschmidt-Leitz, E., and Kustner, G., *Physiol. Chem.*, 1927, **171**, 70.

[12] Bucher, G. R., and Beazell, J. M., *Am. J. Physiol.*, 1941, **133**, 230.

[13] Rose, W. C., *Arch. Int. Med.*, 1913, **5**, 459.

[14] Hackett, W. R., *Lancet*, 1943, **1**, 430.

[15] Anson, M. L., and Mirsky, A. E., *J. Gen. Physiol.*, 1932, **16**, 59.

[16] Cobb, P., *Am. J. Physiol.*, 1905, **13**, 448.

[17] Ayer, J. B., Dailey, M. E., and Fremont-Smith, F., *Arch. Neurol. and Psych.*, 1931, **26**, 1038.

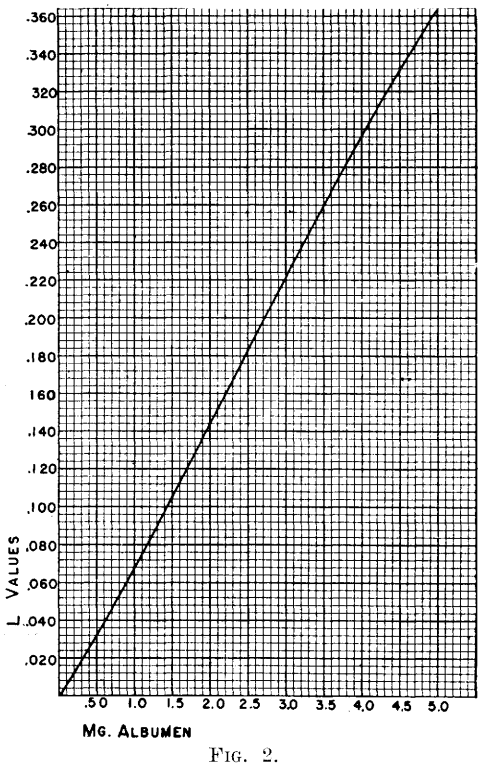

FIG. 2.

The optical density of sulfosalicylic acid precipitated egg albumen, expressed as "L" values, is plotted against the concentration of egg albumen.

tein in cerebrospinal fluid using sulfosalicylic acid as a precipitant and measuring the resultant turbidity quantitatively in a colorimeter. This method can be adapted to the determination of peptic power utilizing albumen as a substrate.

Preparation of Albumen Substrate. "Impalpably powdered, dried egg albumen" (Merck) is dissolved in distilled water so that each cc contains 1 mg. Before diluting to the mark, a few cc of 1/1000 aqueous merthiolate is added as a preservative. A drop of caprylic alcohol may be added to prevent foaming. The solution is placed in a refrigerator for 2 days and then filtered and refiltered until crystal clear (rarely centrifugation is necessary).

The amount of protein nitrogen in solution is only 70% as determined by the Kjeldahl nitrogen method. Since the units are arbitrary, the results are calculated from the weight of the dried powder.

Dried egg albumen is prepared from many sources of eggs and its composition is quite uniform. A small portion of the albumen is denatured in processing but remains insoluble. Nephelometric readings on various fresh lots of dried albumen have yielded almost identical results. In addition, their digestibility varied but slightly. It is believed that this protein is a satisfactory substrate for practical use. (A more sensitive alternate method using crystalline protein will also be discussed).

Solutions prepared as described are stable for many months if kept in a refrigerator. If a slight turbulence develops, the solution can be decanted or filtered from time to time. It is best to change the solutions once a month.

Standardization of Albumen Solution. Density curves are made under the conditions obtained in the performance of the test. The albumen solution is diluted so that 5 cc will contain 0 to 5 mg of albumen.

Five cc of these dilutions are placed in Evelyn photoelectric colorimeter tubes and 4 cc of N/20 HCl is added to each. One cc of .5 N NaOH is added. The total volume is then brought to 24 cc by the addition of 14 cc of 6% sulfosalicylic acid. (The quantities are for direct reading with an Evelyn photoelectric colorimeter: other colorimeters may accommodate but part of the total mixture). After inverting 3 times *(do not shake)* the tubes are read in the colorimeter using a No. 420 filter. If the "L values" for the galvanometer readings are plotted against albumen concentrations, a line is obtained which is almost straight (Fig. 2).

The particle size varies with the concentration of albumen. The readings are a mixture of transmitted light (specular density) and light reflected from particles nearest the photoelectric cell (diffuse density). For these reasons, Beer and Lambert's law does not hold and a straight line relationship between optical density (L value) and concentration of albumen is not to be expected. The practical range over which reproducible nephelometric readings are possible is 0 to 5 mg of egg albumen.

Choice of Dilution for Performance of Test. Bucher *et al.*[10] have indicated that above a 1/100 dilution, augmentation of pepsin ac-

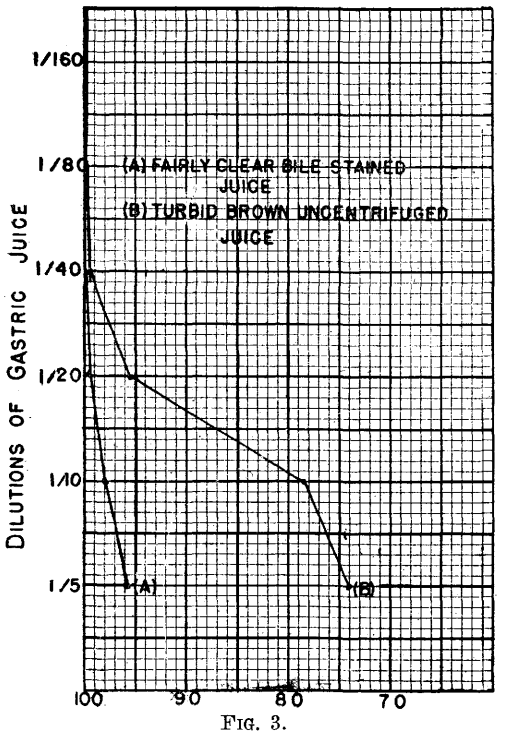

FIG. 3.

The optical density (galvanometer readings only) of gastric juice precipitated with sulfosalicylic acid is plotted. One hundred represents the light transmission of distilled water.

tivity does not occur with further dilution. In rare exceptions, a dilution of 1/200 is necessary. For this reason the actual final dilution in the performance of the test is 1/563.

Choice of Digestion Time. Digestion does not proceed at a uniform rate for more than a brief period (Fig. 1). This interval is a matter of seconds in the described test, rendering accurate measurement most difficult. A digestion time of 30 minutes allows for the greatest and most uniform change.

Choice of Substrate Concentration. A substrate concentration of 5 mg permits the assay of wide variations in pepsin concentration and yet remains within a reasonable range of accuracy for the nephelometric method. Rarely, specimens of canine gastric juice reach the upper limit of the range, but none have necessitated change in method.

Digestion pH. The digestion pH repeatedly determined by the glass electrode is 1.75.

Procedure for Performance of Test. To 5 cc of albumen solution is added 4 cc of 1/250 dilution of gastric juice in N/20 HCl. (The author pipettes 0.2 ml of gastric juice into 50 cc N/20 HCl). The tubes are placed in a constant temperature water bath at 37°C for ½ hour. Digestion is then terminated by the addition of 1 cc of N/2 NaOH. Incubation is continued for ½ hour following which 14 cc of sulfosalicylic acid are added. The alkali and additional period of incubation are necessary for complete inactivation of pepsin, which does not occur with sulfosalicylic acid alone. The sulfosalicylic acid precipitate may otherwise be digested. Densimetric readings are made 15 minutes after the addition of sulfosalicylic acid.

Calculation of Result. The number of milligrams of albumen remaining undigested is determined from the colorimeter reading by referring to the standardization curve. (Fig. 3). This amount subtracted from 5 mg, the amount initially present, yields the number of milligrams of albumen digested by 2/125 cc of gastric juice. The result may be left in this form (peptic power) or converted into pepsin units.

If the mg albumen digested by a known quantity of crystalline pepsin† is plotted on semilog paper, a straight line is obtained; the quantity of albumen digested being proportional to the logarithm of the pepsin concentration. Expressed in another way, the concentration of crystalline pepsin is proportional to the antilog of the quantity albumen digested. The results are merely expressed in the latter proportionality units (designated crystalline pepsin equivalents) because the action of various crystalline pepsins differs.[18]

Illustrative Example. Crystalline pepsin equivalents are obtained by finding the antilogarithm of the milligrams albumen digested using the digits to the right of the decimal point as the logarithm and those to the left as the mantissa.

1. 1.25 mg albumen digested

† Crystalline pepsin was obtained through the kindness of Dr. R. M. Herriot of Rockefeller Research Foundation and by purchase from Plaut Research Laboratories, Bloomfield, N.J.

[18] Wiss, O., *Helvetica Chimica Acta*, 1946, **29**, 237.

Antilog .25 with a mantissa of 1 = 18

2. 3.76 mg albumen digested

Antilog .76 with a mantissa of 3 = 580

Since the figure obtained is for 2/125 of a cc of gastric juice, the pepsin equivalents must be multiplied by 62.5 to obtain pepsin units per cc. To complete the example:

1. Pepsin unit of 18 × 62.5 = 1125
2. Pepsin unit of 580 × 62.5 = 36,250

Reproducibility. Twenty-five separate determinations of mg albumen digested were made using single samples of artificial canine and human gastric juice. The mean deviation was in the neighborhood of 2% in each case. The greatest deviation in any one sample was 5%.

Sources of Error. The density of the gastric juice (which contains protein itself) was determined in various dilutions by precipitation with 6% sulfosalicylic acid. (The same quantity of gastric was used as in the performance of the test except that distilled water was substituted for albumen solution). With uncentrifuged turbid gastric juice, there is practically no optical density attributable to gastric juice alone at a dilution of 1/250 (Fig. 3). In rare cases, where the gastric juice is alkaline, a sufficient quantity of mucus may be in solution to influence the densimetric readings. In such cases it is a good practice to do a blank determination as described above and subtract the optical density due to mucus. Some gastric juice may be bile colored—at 1/250 there is no evidence of color. If the juice is very highly colored a blank may be prepared from the diluted juice by substituting distilled water for albumen.

Sulfosalicylic acid occasionally takes on a slight brown color. In this case, a water blank should not be used but a blank containing 10 cc of water and 14 cc of 6% sulfosalicylic acid.

Failure to obtain good mixture at any stage of the procedure can produce a large error. Failure to invert the tubes several times before reading can produce a considerable error.

If greater accuracy is desired in assaying juices of extremely low peptic activity, half concentrated substrate should be used. This dilution puts the nephelometric determination in its most sensitive range.

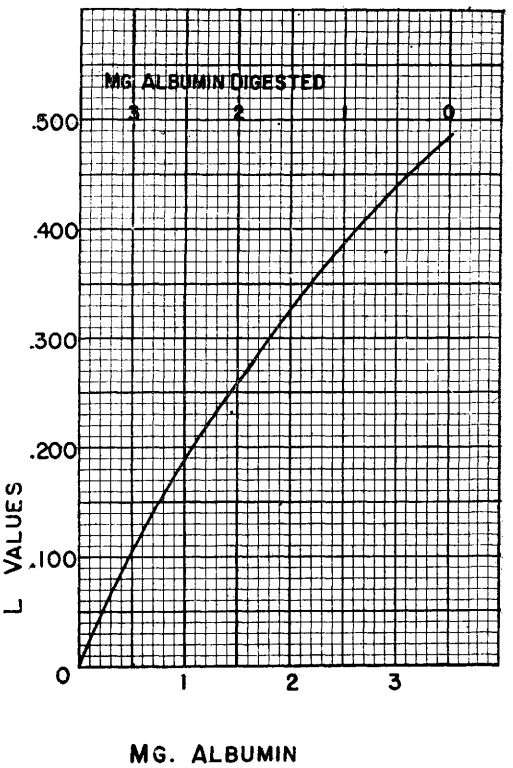

FIG. 4.

The optical density of sulfosalicylic acid precipitated albumin, expressed as "L" values, is plotted against the concentration of human serum albumin.

Alternative Method Using Purified Bovine or Human Albumin.‡ The nephelometric curve for human and bovine albumin is shown in Fig. 4. (The curves for human and bovine albumin are almost identical and may be so considered for practical purposes). The workable range for nephelometric determinations is from 0 to 3.5 mg under the experimental conditions given.

Human or bovine albumin is dissolved in distilled water so that each cc contains 2.1 mg.

One-tenth cc of gastric juice is diluted to 50 cc with N/20 hydrochloric acid. Five cc of diluent is mixed with 5 cc of albumin

‡ The highly purified human albumin was obtained through the courtesy of Dr. E. J. Cohn of the Harvard Medical School Serum Laboratory. The highly purified bovine albumin was obtained through the courtesy of Armour and Co. The fact that these materials will probably be available for general use in the near future makes their use ideal.

solution. Digestion is performed for 20 minutes at 37° C, at the end of which time the pepsin is inactivated by the addition of 2 cc of .4 N NaOH and incubation for 30 minutes at 37°C. Thereafter, 4 cc of the inactive mixture is pipetted into a colorimeter tube and 20 cc of 6% sulfosalicylic acid is added. After 15 minutes the optical density is read in the photoelectric colorimeter with a No. 420 filter.

Since an aliquot of ⅓ is taken for the albumin determination, the mg albumin digested as obtained from Fig. 4 are multiplied by 3 to obtain the actual milligrams of pepsin digested.

When the mg albumin digested is plotted against the concentration of crystalline pepsin on semilogarithmic paper, a straight line is obtained. The range of this test is from 5 to 2000 γ. The antilogs of 0 to 3.5 would cover this range without distortion so that the antilog of the mg albumin digested unmultiplied by 3 is used to convert to pepsin equivalents.

It will be noted that the range of this procedure is much smaller than that utilizing egg albumen. Hence it may be occasionally necessary to use a greater initial dilution than is indicated. Although its range is smaller its sensitivity is correspondingly greater.

The digestion pH for this test has been found to be 1.75 as determined by the glass electrode.

To assay juice of extremely low peptic power it is more accurate if half concentrated albumin is used for the substrate.

Summary and Conclusion. The disadvantages and inaccuracies of many tests for pepsin have been discussed. A method for determining total pepsin which does not embody these disadvantages is described. Egg albumen is used as substrate and a photoelectric colorimeter used as a densimeter is employed to determine the amount of albumen solution digested. An alternative method of greater sensitivity is also described utilizing purified bovine and human serum albumin. Little technical skill is required and the execution of the tests is exceedingly rapid.

Thanks are expressed to Dr. William H. Fishman for his aid in the preparation of this manuscript.

15568

Active Pepsin Fraction, Resting Peptic Activity, and Percentage of Pepsin Inhibition in Gastric Juice.*

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Total pepsin is only an index of the secretory activity of the pepsinogenic cells and gives no indication of the digestive action *in vivo* as modified by inhibitory substances and pH. Because of the importance of pepsin in experimental ulcer formation[1,2] one should know the quantitive effects of both inhibitory substance and pH.

Total pepsin can be assayed only after high dilution of gastric juice, whereupon it is completely freed from inhibitory peptones. If gastric juice were assayed in its undiluted state, only that portion of pepsin uncombined with inhibitor (active pepsin fraction) would be measured.

If digestion is carried out at different pH's, a correction chart for varying digestion pH's can be constructed. By this means, the actual digestive power of the juice can be obtained after ascertaining the pH of the gastric juice.

The digestive action of juice corrected for pH and inhibitor substances has been designated resting peptic activity, and is a measure

* Done on a grant from the Otho S. A. Sprague Memorial Institute.

1 Schiffrin, M. J., and Warren, A. A., *Am. J. Dig. Dis.*, 1942, **9**, 205.

2 Driver, R. L., Chappell, R. H., and Carmichael, E. B., *Am. J. Dig. Dis.*, 1945, **12**, 166.