

somewhat sticky yellow powder which on slow heating started to soften at 67° and melted completely at 125°. It contained N 2.2%, P 3.4%.

From the defatted bacteria a *polysaccharide* was obtained which amounted to about 3.5% of the material. This substance contained 65.3% of reducing sugars (calculated as glucose).

A detailed report on the chemical composition of the fractions mentioned and on their biological behavior will be presented at a later date.

The various substances isolated are being tested, at present, as to their effect on the Paris daisy, castor bean, geranium, Bryophyllum and two species of tobacco. Preliminary tests made on the Paris daisy, geranium and Ricinus indicate that the phosphatide acts as a growth-stimulant, whereas the fat is much less active.

8748 C

Some Physical and Chemical Properties of Desiccated Stomach.

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Since the introduction of the use of desiccated hog stomach in the treatment of pernicious anemia by Sturgis and Isaacs,¹ Sharp,² Conner,³ and Wilkinson,⁴ efforts have been made to fractionate the material and determine the nature of the active principle. In the following experiments whole hog stomach was finely ground, dried at a temperature below 30°C., and the fat extracted with petroleum benzine. With this method 100 gm. of fresh stomach yield 14-15 gm. of dry powder. This material is stable, retaining its anti-anemic potency for months. It is non-hygroscopic but on the addition of water swells and forms a milky suspension.

This material was used as a starting point for further manipulations. In each case the substance was given to one or more suitable patients with pernicious anemia having the following qualifications: a macrocytic anemia with a red blood cell count of less than 2.5 millions per cubic millimeter; no free hydrochloric acid in the

¹ Sturgis, C. C., and Isaacs, R., *J. A. M. A.*, 1929, **93**, 747.

² Sharp, E. A., *J. A. M. A.*, 1929, **93**, 749.

³ Conner, H. M., *Proc. Staff Meet. Mayo Clinic*, 1929, **4**, 319.

⁴ Wilkinson, J. F., *Brit. Med. J.*, 1930, **1**, 236.

stomach after histamine injection intramuscularly; no previous anti-anemic medication for at least one month; hospitalization with close observation during treatment; no gross infection; no evidence of inability to absorb material given by mouth (normal gastric emptying time); corroborative clinical data of pernicious anemia.

Studies of the properties of desiccated stomach show that it contains pepsinogen, that the peptic activity (Mett's test) is absent in fresh aqueous filtered solutions, and that the aqueous filtrate of 5 gm. of desiccated stomach in 50 cc. of distilled water develops an activity equivalent to 0.5% pepsin when acidified with HCl.

A 1:100 aqueous extract of desiccated stomach dried in open pans in the air current from an electric fan (5.13 gm. = 40 gm. of desiccated stomach tissue) gives the calculated hematopoietic response when fed to a patient with pernicious anemia. The dried aqueous extract retains its peptic activity but is inactivated after treatment with acetone. Prolonged contact with acetone inactivates both the pepsin and the hematopoietic property, the degree of inactivation being proportional to the time of contact. Ultrafiltration (pyroxylin-Berkefeld filter) also removes the peptic activity and the hematopoietic activity.

A watery suspension acidified to pH 4.5 with HCl gives a clear yellow filtrate which is as active in the treatment of pernicious anemia as a dried aqueous extract. This acid extract also has peptic activity. Heating an aqueous suspension or an acid extract of stomach to 60°-70°C. for half an hour inactivates it for both its peptic activity and its ability to induce a hematopoietic remission in a patient with pernicious anemia.

Although the peptic activity and the hematopoietic activity parallel one another, specimens of desiccated stomach inactivated by heat do not regenerate their hematopoietic activity after incubation with commercial pepsin.

In determining the effect of alkali upon the active principle, 500 gm. of desiccated stomach were suspended in 5 liters of water, made alkaline with NaOH to pH 9.0, and stirred for an hour. The mixture was filtered and the filtrate, after neutralization to pH 7.0, was fed daily in 200 cc. amounts to a patient with a red blood cell count of 1.38 millions per cubic millimeter producing a maximum reticulocyte response of 25% in 7 days.

It is known that fresh human gastric juice alone does not produce a response when fed to a patient with pernicious anemia,^{5, 6} so

⁵ Castle, W. B., and Townsend, W. C., *Am. J. Med. Sci.*, 1929, **178**, 764.

⁶ Helmer, O. M., Fouts, P. J., and Zerfas, L. G., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 775.

desiccated stomach and its potent extracts must supply another factor in addition to the enzyme-like substance contained in gastric juice. The following experiment was planned to determine the nature of the other factor which with the enzyme-like substance is the active principle of the stomach tissue.

When an acid extract (pH 4.5) of 400 gm. of desiccated stomach was boiled for 5 minutes the clear yellow solution became cloudy but did not coagulate, so it was concentrated to a small volume, made 60% alcoholic, and filtered. The filtrate was concentrated under reduced pressure to remove the alcohol and 40 cc. (representing 56 gm. of desiccated stomach) were fed daily to a pernicious anemia patient, with no hematopoietic response. After 7 days 40 cc. of the same material were incubated for 3 hours with 200 cc. of human gastric juice and fed to the same patient at 8 o'clock every morning, breakfast being omitted. The red blood cell count was 1.43 millions per cubic millimeter and the number of reticulocytes was 15.3% in 9 days. It is thus evident that a second substance necessary for the development of the anti-anemic principle is present in stomach tissue. This substance is heat-stable and probably interacts with the heat-labile factor in a manner similar to the behavior of the "extrinsic" (heat-stable) and "intrinsic" (heat-labile) factors postulated by Castle and Strauss.⁷

The next series of experiments was designed to determine the effect of incubation on stomach extracts and to prepare products suitable for intramuscular injection.

An acid extract of desiccated stomach was made 70% alcoholic, evaporated to a small volume *in vacuo*, and sterilized by filtration through a Berkefeld filter. Such a preparation, without the sterilization, had been inactive when administered orally and it was desired to determine its activity when injected. The equivalent of 120 gm. of desiccated stomach was given intramuscularly over a period of 5 days to a patient with no hematopoietic response.

An acid extract was incubated alone for 8 hours, made 70% alcoholic, and after the alcohol was removed the solution was boiled and evaporated to a small volume. The equivalent of 175 gm. of desiccated stomach was given intramuscularly over a period of 7 days to a patient with no hematopoietic response.

At this time we observed that Wilkinson and Klein⁸ had prepared an active intramuscular product, which was soluble in 70% alcohol and which would resist a temperature of 60°-65°C. for 30 minutes,

⁷ Castle, W. B., and Strauss, M., *New Eng. J. Med.*, 1932, **207**, 55.

⁸ Wilkinson, J. F., and Klein, L., *Lancet*, 1933, **2**, 629.

by incubation of a stomach product with beef muscle. It appeared then that our preparation might also resist a temperature of 60°-65°C., although not a boiling temperature.

Consequently, an acid extract was incubated 8 hours, heated at 60°-65°C. for 30 minutes, made 70% alcoholic, and worked up into a product suitable for intramuscular injection. When the equivalent of 75 gm. was administered to a patient over a period of 3 days the reticulocytes rose from 4.5% to 15.9% in 7 days (R.B.C. = 1,400,000 per cu. mm.).

From these experiments it is evident that the incubation of an acid solution of stomach tissue alone produces a substance which is not identical with the active principle of liver (because it is destroyed by boiling) but which is possibly some intermediate product between the original principle in stomach and the heat-stable principle found in liver.

Summary. A water extract of desiccated hog stomach acidified to pH 4.5 is active in the treatment of pernicious anemia. The hematopoietically active substance does not pass through the ultra-filter and is stable toward alkali. Stomach extracts inactivated by heat do not regenerate their hematopoietic activity by treatment with pepsin. An acid extract contains 2 factors, one heat-stable and the other heat-labile, both of which are necessary for the production of a reticulocyte response in a patient with pernicious anemia. An acid extract incubated for 8 hours produces a hematopoietically active substance which is soluble in 70% alcohol and which resists a temperature of 60°-65°C. for 30 minutes, but not a boiling temperature. Incubation in this manner produces a substance which is not so heat-stable as the anti-anemic principle in liver but which may be an intermediate product more heat-stable than the original stomach fractions.

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Response of the Iris to Pitressin and Pitocin.

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Posterior pituitary extract (pituiratin) has been reported¹ to cause a dilatation of the pupil when dropped into the conjunctival sac of

¹ Pollock, W. B., *Brit. J. Ophthalmol.*, 1920, 4, 106.