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A rapid method of preparing the anti-diabetic substance of pancreas.

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As reported[1] at the last meeting of this branch, heating to 75° and 80° C. for one-half hour does not destroy the anti-diabetic substance extracted in acid aqueous media. The original observation of Murlin and Kramer that a potent extract could be prepared by boiling dog's pancreas in 0.2N HCl was confirmed[2] some time ago. Recently we have compared the yield of rabbit units obtained on the one hand by grinding macerated pancreas in 0.2N HCl in a bacteria grinder for 15 hours and, on the other hand, by heating to 75° C. for one hour or bringing just to boiling temperature.

Kimball, Piper and Allen[3] showed also at the last meeting that the active substance is precipitated by complete saturation with sodium chloride, and with either of the alcohols, methyl, propyl, butyl or amyl when added to a solution in 80 per cent. ethyl alcohol. For example, preparation No. 140, heated in 0.2N HCl to 75° C, cooled, strained through cheese cloth, neutralized to P_H of 4.1, and filtered, gave in two tests representing 10 gms. pancreas each, a fall of blood-sugar in normal rabbits of 44 and 62 milligrams. The second day the filtrate was precipitated by saturation with sodium chloride, the precipitate taken up in 70 per cent. alcohol, centrifuged to remove insoluble protein and reprecipitated with 5 vols. amyl alcohol. The excess of amyl alcohol was evaporated off before a fan and the residue taken up in sterile water. Equivalent doses representing 10 grams pancreas now gave drops in blood-sugar in rabbits of the same size of 77 and 74 milligrams showing that all the potency had been re-

1 Piper, H. A., Mattill, H. A., and Murlin, J. R., PROC. SOC. EXP. BIOL. MED., 1923, xx, 413.

2 Murlin, J. R., Clough, H. D., Gibbs, C. B. F., and Stokes, A. M., *Jour. Biol. Chem.*, 1923, lvi, 253.

3 Kimball, C. P., Piper, H. A., and Allen, R. S., PROC. SOC. EXP. BIOL. MED., 1923, xx, 414.

moved. The filtrate from the NaCl precipitate contains no active substance. The rapid method which, even with filtration over night, can be carried to completion in 18 hours is as follows:

1. Beef pancreas trimmed free of extraneous tissue at the slaughter house is placed at once in 0.2 N HCl, chilled to 0° C., and is transported to the laboratory in this condition.
2. Upon arrival at the laboratory the acid is discarded, the pancreas hashed in a meat grinder and a known weight mixed immediately with 4 vols. fresh 0.2N HCl.
3. The mixture is brought to 75° C. where it can be held for one hour. Or it may be brought rapidly over a free flame just to the boiling point.
4. It is then chilled under the tap to 20° C. or lower to congeal the melted fat which is skimmed off.
5. The material is strained through cheese cloth and neutralized with N/1 NaOH to a P_H of 4.9 or titration to phenolphthalein to .01 N. It is then filtered through coarse filter paper over night.
6. To each 1,000 c.c. of filtrate 250 grams NaCl are added and stirred to complete solution. Precipitation is rapid and complete. The precipitate contains all the active substance together with some extraneous proteins.
7. After standing for at least two hours the suspended precipitate is decanted and either filtered or centrifuged off.
8. In either case the precipitate is treated with alcohol of not over 70 per cent. by volume and the insoluble proteins discarded.
9. Three to five volumes of amyl alcohol are added and the mixture thoroughly shaken and centrifuged. The precipitate is found between the aqueous and alcoholic layers.
10. The precipitate is treated with 80 per cent. alcohol, filtered, and the filtrate evaporated to dryness by air currents. Further purification is accomplished by re-solution in 80 per cent. alcohol and evaporation *in vacuo*.
11. The dry material is taken up in sterile water and filtered aseptically, adjusting the reaction of P_H of 4.0 or lower.
12. This final product usually gives a very faint biuret reaction.

The final product is water clear and, because the final precipitate is very soluble in water, can be made as concentrated as desired for administration.

For treatment of perfustates[1] made with 0.2 per cent. HCl and improved extracts recently prepared by percolation, neither of which contains much protein, the method of refinement yielding best results is as follows:

1. Excess of acid is neutralized to P_H of 5.85. Acid metaproteins are thrown down. Fluid is filtered and filtrate immediately readjusted to P_H of 4.1.

2. Sodium chloride in the proportion of 1 gram salt to 3.5 grams pancreas used is added to the first filtrate and dissolved. The fluid is then evaporated to dryness.

3. Excess salt and proteins are left behind by successive fractional extractions with 80 per cent. alcohol and evaporation to dryness.

4. The final residue is treated with a small volume of sterile, distilled water and the reaction which is now about N/5 HCl, readjusted to P_H 4.1 (15 c.c. fluid = 2.1 c.c. N/10 NaOH to phenolphthalein). *The anti-diabetic substance is precipitated in a form which is insoluble in sterile distilled water but is readily soluble in weak acid or weak alkali.*

5. It is free of chlorides and gives none of the following reactions for proteins: Biuret, Millon's, Xantho-proteic and Hopkins-Cole.

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The fate of iletin in the animal body.

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Fasting animals show a different counter regulation for iletin than animals which received food. Rabbits with food plus iletin may show lowering of the blood-sugar for periods of eight hours. Convulsions have been observed as long as 14 hours after the iletin injection. This indicates that iletin is not rapidly destroyed or eliminated by the animal body. No change in the tolerance for iletin was observed after a daily administration of the extract for 83 days.

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[1] Murlin, J. R., Clough, H. D., Gibbs, C. B. F., and Stone, Neil C., *Amer. Jour. of Physiol.*, 1923, lxiv, 348.