

tive; the August material happened to be the most active one, while the winter and June material were about of equal strength.

Conclusions. We may conclude that seasonal variations corresponding to those which have been observed in the mammalian thyroid gland could not be established in the case of the anterior pituitary of cattle. While these investigations do not absolutely exclude the possibility that cyclic changes of a more subtle kind may occur in this organ, they render them at least very improbable. It is, therefore, probable that the seasonal variations in the thyroid gland are not determined by primary changes of an annual cyclic character in the anterior pituitary, but that they are due to climatic differences during the seasons of the year, and that the effects of these differences are transmitted to the thyroid gland either directly from the periphery or indirectly through the mediation of other organs. The seasonal changes in the thyroid gland apparently correspond to differences in the need of thyroid hormone at different times of the year. Owing to a diminished need of thyroid hormone on the part of the organism during the summer months, after extirpation of the greater part of the thyroid the remaining part of the gland responds less actively with compensatory hypertrophy during this time of the year.

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Simple Method for Obtaining Antisera in a Dry State.

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Several reports deal with the preparation of dry antisera. Evaporation *in vacuo* and precipitation in the cold with alcohol or acetone are the methods most used. According to reports dealing with the latter method, the low temperature has been assumed to be the factor preventing denaturation and loss of antibody activity. We have found that the concentration of the organic precipitant is an equally important factor. There exists a critical concentration of the organic solvents methyl, ethyl and propyl alcohol and acetone, in the range of 60% to 75% concentration at which denaturation of serum proteins is maximal. As the concentration is increased from about 75% the degree of denaturation is decreased until at final concentrations of 90% or above serum proteins can be precipitated

by these organic solvents at room temperatures without loss in solubility or antibody activity.

Applying this phenomenon the following method has been used to prepare dry immune sera. To 10 or more (but not less) volumes of acetone add slowly with shaking one volume of the serum. Collect the precipitate on a filter, wash once with acetone followed by 3 washings with anhydrous ether, the precipitated mass being stirred with a wooden spatula after each ether addition. Approximately 5 volumes of ether to each original volume of serum is required for each washing. The final white mass is spread out on the filter paper and placed in the 37°C. incubator for about one hour. The resulting dry mass is readily pulverized with the wooden spatula to an extremely light, white, fluffy powder. This powder is slowly (due to slow wetting) though completely soluble in distilled water or physiological sodium chloride solution. There has been detected no loss in agglutinating activity, hemolytic activity or antitoxin content.

Absolute ethyl alcohol may be substituted for acetone in the above procedure. Ninety-five percent alcohol may also be used but 19 volumes of alcohol to one of serum are then necessary so the final alcohol concentration does not fall below 90%. It is essential to use anhydrous ether. Extraction with U.S.P. or anesthesia ether yields a final product that is granular and slightly brown in color. Even this product, however, is completely soluble.

The final product appears to be dissolved a little more quickly if 95% or absolute alcohol is substituted for the acetone used for washing in the method given above. The order in such a case is acetone precipitation, alcohol extraction, followed by 3 ether extractions.

The above methods of precipitation and washing have been found to be satisfactory at all temperatures below 35°C. Following precipitation the serum proteins must not be left in contact with the organic solvent longer than about 4 hours if the final product is to be completely soluble.