

from the fry of the treated group was found to be small, and limited to the region immediately about the ventral aorta. The alveoli were small and distended with stainable colloid material, and the epithelium was of a very low cubical type.

The alveoli of the fry not receiving iodine and seemingly normal were slightly larger than the alveoli of the fry having received iodine. Likewise, the alveoli were found to be only incompletely filled with stainable colloid material, the walls smooth and free from plications and the epithelium of a cubical type. Gaylord and Marsh describe the thyroids of the trout from Michigan and Wisconsin as being slightly hyperplastic. The fry not receiving iodine, and that died, showed the same microscopic conditions as the exophthalmic fry, indicating that the hyperplastic thyroid was more common than the normal thyroid in the control group.

From the present study it is concluded that iodine prevents the appearance of exophthalmia in trout-fry. The minimum iodine required to prevent the appearance of exophthalmia has not been determined. The iodine as total iodine from the water from the spring stratum was found to be 8.9 parts per billion parts of water. Since very few died that received iodine, it is assumed that the fry not receiving iodine, and that died without exhibiting exophthalmia, died from the same cause for they have been found to lack only the one characteristic. Iodine causes a decrease and a disappearance of exophthalmus, involution of the thyroid gland, the appearance of colloid material in the alveoli as well as an increase in physical vigor. Since the characteristics of the disease do not recur as long as they receive iodine, indications are that it is due to a lack of iodine.

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4137

### **Effect of Foreign Protein and of Insulin Administered by Mouth After Ox-Gall.**

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While in the midst of a series of agglutination and precipitin tests on rabbits and guinea pigs the writers came across several news

items announcing the success of Professor A. Besredka and his assistant, Dr. Harry Plotz, in securing immunization against typhoid, dysentery and cholera through administering the appropriate vaccine by mouth after a preliminary dose of ox-bile. It seemed worth while to determine whether or not precipitin reactions could be induced by similar treatment and to test the possibility of thus administering insulin. The following experiments were made.

*Precipitins.* Since experiments were already in progress with antibodies developed against *Beta crystallin* prepared from the lens of the calf, an extract of this protein was used in these tests. In every case, before treating the animals, samples of their blood were taken and a lens precipitin test was made with the serum, using a 1 to 40 dilution of *Beta crystallin* as antigen. The tests were invariably negative. The rabbits were next kept without food for 24 hours, then inspissated ox-gall diluted with 10 volumes of water was administered by means of a stomach tube. Following this, *Beta crystallin* was similarly administered. This procedure was repeated every third or fourth day until 3 such "feedings" were given. Ten days after the last treatment samples of blood were taken and precipitin tests made on them.

Eleven rabbits were used. Of the 9 to which both ox-gall and protein were administered, one died before the final test was made. The serum of the 8 remaining rabbits when tested with a 1 to 40 solution of *Beta crystallin* gave positive titers varying in different animals in dilutions of from 1/320 to 1/5120. The 2 controls, one given protein without the gall, the other gall without the protein, remained negative. In these absorption tests the amount of protein used was about double that ordinarily employed in developing precipitin reactions by intravenous injections.

*Insulin by Mouth.* When insulin is injected into the blood stream of a normal rabbit it produces a characteristic shock in the animal and the blood sugar falls markedly inside of 2 hours. Using the foregoing method, ox-gall and insulin were administered to 3 rabbits. The blood-sugar of the animals was determined before being fed, then again 2 to 8 hours after, but no perceptible reduction was found. Neither did they display insulin shock. When 6 guinea pigs were similarly treated, however, 5 of them died within 24 hours after treatment, while one, although given ox-gall and insulin 5 times, showed no ill effects. Those which died began to tremble soon after feeding, their hair became ruffled and they refused to eat. The controls, one given gall alone, one insulin alone, remained wholly normal.

*Conclusions.* Administration of a lens protein (*Beta crystallin*) by mouth following similar administration of ox-gall resulted, in rabbits, in the production of lens precipitins. Insulin similarly administered yielded negative results in 3 rabbits but gave positive shock effects in 5 out of 6 guinea pigs.

## 4138

## A Method for Determination of Lipin Phosphorus.

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Interest in the *lecithin* content of blood in syphilis necessitated an investigation of the various procedures for the estimation of phosphorus<sup>1-8</sup> or more specifically, lipin phosphorus.<sup>2-6, 9</sup> It was found that "test tube" oxidations by  $\text{H}_2\text{SO}_4$  and  $\text{HNO}_3$  had to be discarded. The danger of bumping with the small amount of reagents used became a source of great concern for micro-determinations.<sup>2-5, 7, 8, 11</sup> This led to the use of  $\text{H}_2\text{SO}_4$  and  $\text{H}_2\text{O}_2$  (suggestions of Baumann<sup>11</sup> and Briggs<sup>3</sup>). Digestions were greatly facilitated, but certain difficulties were encountered. The figures obtained were not constant and often too high, the variations depending on the amount of  $\text{H}_2\text{O}_2$  employed.<sup>6</sup>

After much manipulation, a satisfactory technique was evolved, which may be detailed as follows:

Blood (0.5 cc.) is pipetted into 10.0 cc. of an alcohol-ether mixture (3:1) contained in a 50 cc. volumetric flask, preferably fitted

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