

and 12 of the 24 showed values of 20 mg. or higher.* These were the 12 animals who died immediately after the administration.

The serum phosphorus was, generally, not affected by these very marked increases in serum calcium.

The increase in serum calcium was, roughly, correlated to the dose given, although in some cases a marked increase of serum calcium was obtained after the administration of only a small amount of calcium by mouth. There seemed to be no difference in the effect of the gluconate and the chloride.

As it seems possible that the above results may be due to hyperparathyroidism, we are now determining the amounts of parathyroid hormone in the serum of normal and rachitic rabbits.

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Formation of Antibodies in Fertile Hens Eggs.

E. GEBAUER-FUELNEGG. (Introduced by C. A. Dragstedt.)

From the Department of Research Bacteriology, Northwestern University Medical School.

The theoretical and practical interest inherent in the question of the time and location of antibody formation in the developing organism is responsible for a series of experiments in which fertile hens' eggs were repeatedly injected with foreign sera. After incubation for a certain time, the eggs were opened and precipitin tests were made on the remaining egg white. These tests were positive in approximately 4% of the cases.*

The handling of the eggs during the process is quite delicate, and in spite of careful manipulation, a number of eggs were lost. Because of the obvious necessity of insuring a well-growing chick, the best grade of hatching eggs must be procured. The first injection is usually made on the third day of incubation, when fertility may be ascertained. The egg shell was first washed with mercuric bichloride and then with alcohol. Into each egg, from 0.1 to 0.25 cc. of sheep serum [foreign soluble protein] which had previously been passed through a Berkefeld filter, was injected. In piercing the shell, care must be taken to cause as little disturbance of the con-

* 5 animals had a serum Ca of 20-29, 5 of 30-39, and 2 over 40.

* Young animals are poor antibody producers. R. H. Jaffé, *Physiol. Rev.*, 1931, 11, 279.

tents as possible. The same precaution must be observed while forcing the foreign protein out of the syringe. After the injection had been made, the needle was retracted, and the hole immediately sealed with fairly thick collodion. These injections were repeated 3 or 4 times at intervals of 2 to 3 days, and the eggs were continuously incubated at 37.5°C , and handled as they would be for ordinary hatching purposes. After a total of 14 days' incubation, the eggs were opened and the egg white removed. It was diluted with 5 volumes of physiological salt solution, and filtered until perfectly clear.

Precipitin tests were made in two series: (1) the egg white concentration was kept constant and the antigen diluted, (2) the egg white was diluted and the antigen concentration kept constant. All dilutions were made with physiological salt solution. Controls included normal egg white, serum of another species, and hypo- and hypertonic salt solutions. From 126 fertile eggs successfully handled in this manner, 5 gave positive precipitin tests. The highest titre observed was 1 : 500 [antigen diluted], and the lowest 1 : 50.

Attempts made to hatch chicks from eggs repeatedly injected with foreign proteins were found to be successful in a number of cases, particularly when the injections were begun at a later stage of incubation. As an insufficient number of such animals have been tested up to the present time, no definite statements may be made, but it was observed that antibody formation was apparently obtained in a low percentage. The same holds true for experiments of passive transfer into other test animals of egg white in which the presence of antibody had been previously demonstrated.

A general discussion of the results must be postponed. One minor aspect deserves mention. That antibodies can be produced offers support to the conception that the egg contents are at least partly or as a first step absorbed before utilization by the embryo. The presence of antibodies in the remaining egg white may be attributed either to permeability of the vessels concerned, or to the difficulty of separating the egg white proper from the circulatory channels and their blood content.