tions was 334 beats per minute. There was, therefore, no significant effect of the aconitine on heart rate. Similar results were obtained with 3 rats under amytal anesthesia.

Following urethane one rat was given 0.0128 mgm. and 3 others were given 0.0256 mgm. of aconitine. The heart beat became irregular in each of these animals.

Experiments were made to determine the minimum fatal dose of the alkaloid. No anesthetic or sedative was used. Two adult rats received 0.0256 mgm. and died within 2 hours. Two rats receiving 0.0192 mgm. and 2 rats receiving 0.0227 mgm. developed serious respiratory and heart disturbances but recovered. These data indicate that 0.026 mgm. is a lethal dose for the rat, while the minimum fatal dose is approximately 0.025 mgm.

The effect of the anesthetic, urethane, on aconitine poisoning was studied by reversing the order of administration, the aconitine being given first. In 2 animals a time interval of 15 minutes was allowed between the administration of aconitine and urethane, and in 2 others the time interval was 30 minutes. The dosages were 50% more than the lethal dose without urethane. Although symptoms of aconitine poisoning occurred as indicated by respiratory and heart reactions, all of the animals recovered.

From these experiments it is evident that urethane anesthesia exerts a definite effect in preventing death from what would otherwise be lethal doses of aconitine.

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Precipitin Production with Phosphorised Caseinogen.

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In a recent paper, Rimington and Kay[1] suggested that an ester linkage existed in caseinogen between phosphoric acid and some other constituent of the molecule. Still later Rimington[2] attempted to find out whether or not still further quantities of phosphorus could be induced to combine with the protein. The author succeeded in phosphorizing caseinogen and sero-globulin by means of

[1] Rimington and Kay, *Biochem. J.*, 1926, xx, 777.

[2] Rimington, *Ibid.*, 1927, xxi, 272.

phosphorus oxychloride in a manner analogous to the Schotten-Baumann reaction for the benzoylation of organic substances in an aqueous solution, thus avoiding the risk of incurring destructive changes in the protein. In the case of caseinogen he found 1.77% P, corresponding to a ratio of P/N = 0.130.

Through the courtesy of Dr. Rimington we have obtained some of the native phosphorized caseinogen to ascertain whether or not fundamental changes have taken place in the immunological properties of the new product. From the standpoint of "specificity" observations of this type are of fundamental interest and the more so when destructive changes in the preparation of the protein have been greatly eliminated.

One great difficulty encountered in this study was the low solubility of the native caseinogen, the phosphorized product being much more soluble, giving a thick, viscid solution. One gram of the products as ground up in a sterile mortar and distilled water was gradually added together with N/10 NaOH until the pH was 8.0. The total volume was then made up to 100 ml. By weighing it was found that the caseinogen solution was 1 :6500. The phosphorized product, however, was readily soluble.

Nine rabbits were given intravenous injections of from 5 to 10 mls. every 3 days and the injections were continued longer in the case of the caseinogen (12 against 9). When signs of shock were observed, showing that both proteins were effective as sensitizing agents, intraperitoneal injections were given. Following the injections, a rest period of about one week was allowed before a bleeding was made and the animals were given no food for a period of 24 hours prior to the bleeding. A series of 6 animals died during the course of immunization with caseinogen.

Caseinogen induced the formation of precipitins generally of low titer, with exception of one animal, the serum of which had a titer of 1 :±6500. This reading was made by means of the ring test in Hektoen precipitin tubes after 2 hours at room temperature. Under the same conditions this serum had a titer of 1 :1000 against phosphorized caseinogen. The phosphorized caseinogen induced the formation of precipitins of higher titer than that induced by native caseinogen. The serum of one animal produced marked precipitation with phosphorized caseinogen in a dilution of 1 :64000 and slight precipitation at 1 :128000. This serum precipitated native caseinogen in a dilution of 1 :26000. Only mild skin reactions were obtained in sensitized animals and the reactions were more marked in the case of the homologous protein. Complement fixation reactions

proved to be difficult because of the high anticomplementary effect of caseinogen but this phase of the problem is being studied.

Summary: Both native and phosphorized caseinogen proved to be precipitinogenic. The reactions occur in higher dilutions when the homologous proteins are employed. Antisera against the phosphorized caseinogen precipitate caseinogen and conversely antisera against caseinogen precipitate the phosphorized caseinogen. Phosphorylation of the caseinogen does not destroy its antigenic character.

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Intracutaneous Vaccination of Rabbits with Pneumococcus. I. Antibody Response.

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Suspensions of heat killed pneumococci were injected into the skin of rabbits at intervals of 7 days during a period of 10 to 14 weeks. The total amount of bacterial substance injected was equivalent to and often greater than that ordinarily employed in the routine immunization of rabbits by the intravenous method. Pneumococci of Types I and III and a degraded "R" strain derived from Type II were used. The sera of the treated rabbits were tested for the presence of agglutinins, precipitins, and protective antibodies. The serum obtained from 85% of the animals immunized intracutaneously with Type I pneumococcus failed to show the presence of any demonstrable type specific antibodies. Virulent cultures of Type I were not agglutinated, nor were solutions of the specific soluble substance from organisms of the homologous type precipitated by these sera even when used in high concentrations. Only rarely did the serum confer any passive protection upon mice infected with a virulent strain of Type I, and in these instances the protective titre was low. In only 15% of animals studied was there any serological evidence of type specific response to repeated intracutaneous inoculation of Type I organisms; in these instances the presence in the serum of specific agglutinins was demonstrable only in low dilutions varying from 1:1 to 1:20.

In terms of its capacity to stimulate the formation of type specific antibodies, Type III pneumococcus is at best a poor antigen. It was to be expected, therefore, that organisms of this type, when in-