rest-solutions and in the precipitates from the elutions has been roughly assayed physiologically as a check for the iodometric estimations.

Our experiments show that relatively large amounts of kaolin are required for the adsorption of insulin. Kaolin apparently is not a very specific adsorbent for insulin and it remains doubtful whether it can be used advantageously in the further purification of insulin preparations. Willstätter's special aluminum hydroxide preparations are sometimes extremely specific and selective adsorbents, depending on the type of preparation and the enzyme. Our preliminary experiments with aluminum hydroxide (preparations B and C according to Willstätter) seem to indicate that it is a more suitable adsorbent for insulin than kaolin.

Further experiments are necessary to establish more fully the behavior of insulin in adsorption and elution. It may be possible that such studies will lead to a practical method for the further purification of insulin.

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Glutathione in blood and its utilization in milk secretion.

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The work reported was originally an attempt to follow free cystine in blood by means of the Folin-Looney[1, 2] method. It was planned, if possible, to repeat with cystine the work done in this laboratory with tryptophane[3, 4] and thus to follow its utilization by the mammary gland in milk secretion, and to study further the changes that may occur in the composition of the blood mixture of amino acids as a result of various changes in diet.

Protein free blood extracts were made as in the amino N de-

[1] Folin, O., and Looney, Joseph M., *J. Biol. Chem.*, 1922, li, 421.

[2] Looney, Joseph M., *J. Biol. Chem.*, 1922, liv, 171.

[3] Cary, C. A., and Meigs, Edward B., *J. Agr. Res.*, 1924, xxix, 603.

[4] Cary, C. A., *Proc. Am. Soc. Biol. Chem.*, Dec., 1925.

terminations in this laboratory.[5, 6] These extracts gave values with the Folin-Looney method equivalent to 4 to 7 milligrams of cystine per 100 cc. of blood, and 85 per cent of this occurred in the corpuscles. This was much higher than expected. Furthermore, when cystine was added to the original blood it was never recovered quantitatively. Of 4 mg. dissolved in the acetic acid to be used in the coagulation of the blood, only 1.36 mg. were recovered. When 100 cc. of the original blood were shaken for half an hour with 100 mg. of cystine, only 5 mg. were recovered. With the Folin and Wu[6] tungstic acid method larger amounts of this color producing substance were found in the blood, but there was no recovery of added cystine. Passing air or hydrogen sulphide through the blood for three hours before coagulation had no effect upon the color given by it or upon the recovery of added cystine. Other methods of deproteinization were tried. The results indicated that the color-producing substance in the blood was not cystine; although, when heated with alkali and lead acetate, it gave a precipitate of lead sulfide, and, after treatment with mild reducing agents, it gave the nitroprusside test.

We suspected that the substance might be glutathione, but Hopkins[7] had been unable to find it in blood plasma, and Tunnicliffe[8] had reported it to be absent from whole blood. They had used the nitroprusside reaction which is none too sensitive and which reacts only with the reduced form. Glutathione is oxidized readily in neutral or slightly alkaline solution; and, although Tunnicliffe showed that it is present mainly, if not entirely, in the reduced form in muscle, liver and yeast, it might well occur largely in the oxidized form in the blood where apparently no precaution was taken to reduce it. We therefore decided to try to isolate it from the blood. Our first attempt failed, although some of the substance giving lead blackening and the nitroprusside test was present up to the very last step.

At this time Abel and Geiling[9] reported the presence of labile S in their preparations of insulin. Best, Smith and Scott[10] had

[5] Cary, C. A., *J. Biol. Chem.*, 1920, xliii, 477.

[6] Folin, O., and Wu, Hsien, *J. Biol. Chem.*, 1919, xxxviii, 81.

[7] Hopkins, F. G., *Biochem. J.*, 1921, xv, 286.

[8] Tunnicliffe, H. E., *Biochem. J.*, 1925, xix, 194.

[9] Abel, John J., and Geiling, E. M., *J. Pharmamacol. and Exp. Therap.*, 1925, xxv, 423.

[10] Best, C. H., Smith, R. G., and Scott, O. A., *Am. J. Physiol.*, 1924, lxviii, 161.

shown the presence of this substance in blood. Although the preparations of insulin that we tried gave a faint Folin-Looney reaction, we were able to satisfy ourselves that it did not occur in our protein free blood extracts.

We then attempted to repeat our effort to isolate glutathione using a much larger quantity of blood than before. While this work was in progress Holden[11] published his paper describing the isolation of glutathione from blood. He actually crystallized out 5 mg. per 100 cc. of blood, which, considering the losses that would occur, would account for practically all the labile S that we found in the blood. Holden used the optical rotation of glutathione to guide him in its isolation, whereas we used the Folin-Looney reaction. Assuming the total reaction that we obtained to be due to glutathione, there would be approximately 10 mg. per 100 cc. of blood. This figure is not accurate. However, with some work the method may possibly be adapted to the quantitative estimation of glutathione in blood.

Early in our work we tried to determine whether the substance with which we were dealing, and which we may now call glutathione, is used in the secretion of milk. We compared its amount in samples of blood obtained simultaneously from the jugular and mammary (abdominal subcutaneous) veins of cows. These samples were practically identical when taken from dry cows; but, with lactating cows, the mammary samples showed approximately 25 per cent less than the jugular. That this difference is not due to a shift in the oxidation-reduction equilibrium between the two forms of glutathione is shown by our failure to influence our results by aerating or passing hydrogen sulfide through the blood. Apparently glutathione is used in the secretion of milk, and thus performs another function besides that attributed to it by Hopkins. Assuming that the substance that is thus utilized in milk secretion is entirely glutathione, one may approximate roughly the amount of it that the mammary gland takes out of the blood daily. It is sufficient to account for all of the glutamic acid in the milk proteins and several times the total S content of the milk. We have no definite data as to the disposition of this excess of S. In the formation of milk fat from phosphatide Meigs Blatherwick and Cary[12] found the excess P returned as inorganic

[11] Holden, Henry Francis, *Biochem. J.*, 1925, xix, 727.

[12] Meigs, E. B., Blatherwick, N. R., and Cary, C. A., *J. Biol. Chem.*, 1919, xxxvii, 1.

phosphate to the plasma of the mammary blood, and this S might be oxidized and similarly returned as sulfate to the blood.

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Immunity to pneumococcus afforded rats by feeding them the germ.

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In December it was reported[1] that rats became resistant to pneumococcus injections if fed on the tissues of other rats which had been killed by the same organism. It was shown that such animals tolerated 1000 or more times the dose of pneumococci that proved fatal for control rats. It was suggested that the living germs present in the tissue being fed might be the real cause of this increased resistance. Attention was also called to the fact that experiments had been done in which pneumococci were fed with the object of seeing whether a similar immunity could be built up in this manner. Our experiments at that time indicated that feeding the cocci from 50 cm. of culture per day to a rat produced a decided degree of immunity. It is our intention in the present report to give a representative experiment (our latest), in tabular form and to show that an immunity almost as good as, if not quite equal to that produced in the tissue feeding experiment, can be produced by feeding live pneumococci. Tests have also been made in which the dead germs have been fed, and are included in the table. The immunity built up by this latter method, is not so good as that obtained with living organisms, when fed in equal quantities.

Each of the rats listed in the accompanying table received the germs from approximately 50 cm. of a 24 hour culture of pneumococcus Type I, daily, for about 20 days. The organisms were grown in meat infusion media. The culture was centrifuged, the

[1] Ross, Victor, PROC. SOC. EXP. BIOL. AND MED., 1925, xxiii, 183-185.