were also found in large amounts in the connective tissue of the ciliary body and choroid. None has been observed, however, in the cells of the anterior iris, Schlemm's canal and retina.

The fat might disappear altogether from the ciliary epithelium 8 to 12 hours after injection, but became abundant in the choroid probably carried there by lymph streams and wandering cells.

A comparative study of fat absorption of the intestinal cell was made side by side. Mice and rabbits were fed frequently with cod-liver oil and killed after 2 to 4 hours. The mucous membrane of the stomach and intestine was removed and treated according to the same technique as used for the ciliary epithelium. The distribution of the fat granules in the intestinal absorbing cells was similar to that of the ciliary columnar epithelium. More significant is the fact that the fat granules were never found in any type of the secretory cells of the stomach and intestine.

The above experiments tend to show that the ciliary columnar epithelium has an absorbing function.

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Preparation of Globin.

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Schulz[1] in 1898 prepared globin, the protein moiety of hemoglobin, by extracting an acidified solution of oxyhemoglobin with a mixture of ether and alcohol. Most of the pigment went into the ether-alcohol layer, leaving the globin in the aqueous layer. Upon neutralization with ammonia the globin was precipitated which was redissolved in dilute acetic acid and dialyzed. This method is very convenient, but the globin thus obtained is not natural, but denatured, globin, since the ether and the alcohol rapidly denature the protein in the presence of the acid.

Hill and Holden[2] have recently succeeded in preparing some globin which is soluble in neutral solution and which may be natural globin. They did not start with pure hemoglobin, but with washed cells. They observed that the stroma proteins, when swollen with ether, had a great power of absorbing hematin, and they utilized this

[1] Schulz, F. N., *Z. Physiol. Chem.*, 1898, xxiv, 449.

[2] Hill, R., and Holden, H. F., *Biochem. J.*, 1926, xx, 1326.

property for the removal of the pigment from the globin. Cell solution was acidified with HCl and shaken with ether and kieselguhr. The mixture was filtered, and the filtrate was neutralized with ammonia to precipitate the denatured globin. This was again filtered, leaving a soluble globin in the filtrate. To minimize denaturation, all these operations were carried out at —2°.

As far as the separation of the pigment from the globin is concerned, the extraction method cannot be surpassed. The stroma proteins remove only a part of the pigment, the remainder being carried down by the denatured globin. Hill and Holden stated that the globin prepared by their method contained up to about 2% of methemoglobin. It would seem that the globin solution may be contaminated also by the stroma proteins. Working with small amounts of cells and "with scrupulous attention to details," Hill and Holden reported a yield of globin amounting to about 40% of the original hemoglobin. Working at 0° instead of —2°, and using the cells of sheep instead of ox blood we have been able to obtain a yield of only a few per cent.

The commendable feature of Hill and Holden's method is the cooling of all solutions. We have found that if the extraction of the pigment with ether and alcohol is carried out in the cold and the denatured globin removed by neutralization and filtration, considerable amount of globin is found in the filtrate. As the result of a certain finding about the hemoglobins and guided by our experience with denaturation of proteins in general, we have worked out a simpler method for the preparation of globin.

Method: Prepare methemoglobin as follows: Dilute 100 cc. of washed sheep cells with an equal volume of H_2O. Add 4 g. of powdered potassium ferricyanide. When the evolution of oxygen has ceased, transfer the mixture to collodion tubes and dialyze until free from ferricyanide. The dialyzed solution is treated with alumina cream to remove the stroma proteins.

Measure (a) 32 cc. of 95% alcohol and 112 cc. of ether into a 250 cc. separatory funnel; (b) 150 cc. of water into a 300 cc. flask; (c) 20 cc. of N/10 NaOH into a test tube. Cool all these to 0° by placing them in an ice bath. Mix 40 cc. of 2% methemoglobin solution with 20 cc. of N/10 HCl and cool the mixture also to 0°.

Pour the acid methemoglobin solution into the ether-alcohol mixture. Remove the separatory funnel from the ice bath and shake vigorously for 30 seconds. Return the funnel to the bath. In one or two minutes the mixture will have separated into 2 layers. The upper layer contains nearly all the pigment, while the lower layer

contains the globin. Remove the funnel from the bath and deliver the aqueous layer into the cold H_2O (b) and mix. Pour the N/10 NaOH into the mixture and mix by a gentle shaking. The denatured globin is precipitated. The precipitate flocks well and is very easily filtered. The filtrate contains the globin in a concentration of about 0.2%. If the ether-alcohol mixture is cooled to —15° instead of 0°, the yield is a little higher. The yield is also higher, if 1% methemoglobin solution is used instead of 2% solution. In that case, the final solution of globin is, of course, more dilute.

To remove the ether and alcohol, the solution is dialyzed against water in the cold. If it is desired to concentrate the solution at the same time, the globin solution may be dialyzed against egg white as described in the following paper.

Discussion. In the preparation of globin Schulz used oxyhemoglobin and Hill and Holden used washed cells which contained, of course, also oxyhemoglobin. We have found that different forms of hemoglobin do not give up the pigment to the ether-alcohol mixture with the same ease. Whereas a 1% solution of methemoglobin gives up all of its pigment when extracted as described above, oxyhemoglobin gives up only about 80% of its pigment under the same conditions. If the aqueous layer in the latter case is shaken with ether-alcohol mixture for a second time, very little pigment is removed, and further extraction will not remove any more pigment. If the oxyhemoglobin is reduced in Van Slyke's gas apparatus (constant pressure form) and the extraction carried out in vacuum, the pigment is completely extracted as in the case of methemoglobin. Carboxyhemoglobin occupies a middle position between the methemoglobin and the oxyhemoglobin. This difference in the behavior of different forms of hemoglobin seems significant, and it will be studied further. For the present it suffices to point out the fact that methemoglobin is the most suitable form of hemoglobin for the preparation of globin.

For the complete extraction of the pigment a certain minimum amount of acid is required. Excess of acid is not desirable, as it will accelerate the denaturation of globin. The amount prescribed in the above method is just enough for complete extraction of the pigment from 1% methemoglobin solution. When 2% methemoglobin solution is used, a small amount of the pigment is left in the aqueous layer. The pigment is carried down, however, by the denatured globin upon neutralization of the acid solution.

The dilution of the acid globin solution with H_2O before neutralization is to decrease the denaturing action of alcohol and ether which are dissolved in the solution.

When a solution of hem (prepared by dissolving hemin crystals in dilute sodium carbonate) is added to an equivalent amount of globin and the mixture is buffered to pH 7.4, the absorption band between C and D ($\lambda = 634$) characteristic of methemoglobin can be seen. In 2% Na_2CO_3 solution the 3 bands of alkaline methemoglobin mentioned by Hill and Holden can be seen. When compared with a natural methemoglobin solution of the same concentration, however, the intensity of the bands given by the globin-hem mixture is very much weaker. In fact, the difference in color between the methemoglobin and the globin-hem mixture in carbonate solution is apparent to the naked eye. The globin solution prepared according to Hill and Holden behaves in the same way as one prepared by our method. We are not prepared to interpret this finding at present, and we are studying further the relationship between hem, globin and methemoglobin.

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Method for Concentrating Protein Solutions Without Denaturation.

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In the preparation of natural globin by the method described in the preceding paper the solution obtained is too dilute for certain purposes. It has been found impossible to concentrate the solution by any of the usual methods without great loss as the globin is very susceptible to denaturation by a variety of agents. Finally a method is found which works well for globin and should be applicable to any protein solution.

The principle of the method is simple, but it does not seem to have been employed as a laboratory technique. The protein solution is concentrated by dialysis against a concentrated solution of another protein or some other substance which cannot diffuse through the membrane. Fresh egg white serves this purpose very well. It is convenient to insert into the tube a rubber stopper with a large hole. The tube is tied to the stopper with a string which serves for hanging the tube.

A collodion tube such as is used for dialysis is prepared. Transfer to the tube a measured volume of water and place the tube in about 10 volumes of fresh egg white. The dialysis is allowed to take place in the ice chest.