RETICULIN AND COLLAGEN. By M. SIEGFRIED.

As Mall[1] does not obtain gelatine by boiling reticular tissue in water, he concludes that it is not white connective tissue. Young[2], on the other hand, succeeds in producing gelatine by boiling it in water and holds this forth as a proof that reticular tissue is white connective tissue. Contrary to both authors I[3] proved that it is split up into gelatin and a heretofore unknown albuminoid reticulin.

In the preparation of reticulin I used the intestinal mucous membrane of pigs and, since one can procure only a small amount of reticulin from one pig, I used the mucosa of 8—17 pigs for one experiment. I subjected these membranes to tryptic digestion, washed carefully in water and alcohol, and freed them from fat with ether in a Soxhlet's apparatus. Hereupon, they were again digested and again the fat extracted with alcohol and ether.

If a small amount of the reticular tissue, which has thus been prepared is boiled in a *large* quantity of water for *24 hours*, it is dissolved and turns into an opalescent fluid, from which a voluminous precipitate will be precipitated by acetic acid. If it is boiled for half-an-hour, it loses its structure and is transformed into a very fine powder. The fluid, which gives all the reactions of gelatin, gives a jelly on evaporation. One difference between gelatin and the solution obtained by boiling reticulin in water is, that the latter gives a large precipitate on addition of acetic acid, while glutin does not. In the preparation of reticulin a part of the undissolved reticulin swells in the solution. The addition of ammonium acetate makes it shrink and precipitates it, leaving a clear solution.

Later, Schwarz[4] under the direction of Hoppe-Seyler obtained from the aorta (ox) a protein, the properties of which corresponded for

[1] *Abhandlung d. math. phys. Cl. d. Königl. Sächs. Ges. d. Wiss.* XVII. p. 299. 1898.

[2] This *Journal*, XIII. p. 332.

[3] M. Siegfried. "Ueber die chemischen Eigenschaften des reticulierten Gewebes." *Habilitationsschrift.* Leipzig. F. A. Brockhaus, 1892.

[4] *Zeitschrift f. physiologische Chemie*, XVIII. S. 487.

the most part with my reticulin. What he got he calls a "reticulin-like substance," because he believes in the probability, that in the aorta the elastic fibres are interwoven with the fibres of reticular tissue.

A short time ago Miss Christine Tebb[1] obtained results different from mine. Her experiments with tendon (ox) have nothing to do with my work, but she also experimented with very small quantities of intestinal mucous membrane. In one of these experiments, she used the mucosa of the intestines of a cat, and in another of a dog. According to Miss Tebb's statement, 9 cats will give 0·27 gr. of reticular tissue after digestion and extraction of the fat (p. 470).

As Miss Tebb used the mucosa of one cat she, therefore, only worked with about 0·03 gr. substance, which should be split off in gelatin and reticulin according to my experiments.

Miss Tebb's arrangement of her experiment differs from mine. In the first place, in that she soaked the tissue over-night in a 1 % solution of sodium carbonate, in order to remove the mucin, which she thought would impede the digestion. There is no ground for this apprehension, for the mucin is dissolved and entirely removed by tryptic digestion. In a separate experiment, I have now convinced myself that the intestinal mucous membrane of a pig which has been digested, etc. according to my directions, is entirely free from mucin.

In Miss Tebb's first experiment the mucosa was digested for 5 days and then boiled 12 times, each time for 20 minutes with 50 c.c. of water. Each of the 12 extracts, which together were obtained from 0·03 gr. of tissue from which the fat was removed, gave a precipitate with tannin. The first six extracts together gave a jelly, the seventh, eighth and ninth extracts together also yielded a jelly; the tenth extract, also the twelfth, each yielded a tender jelly. The remainder was boiled for 2 hours 40 minutes, and then the whole night long. There was a small residue, which gave the xanthoproteic reaction. "This was not reticulin, for according to Siegfried[2] reticulin, when boiled for many hours continuously, forms an opalescent solution."

In reply to this assertion, I wish to remark, that, as regards the solubility of reticulin, I made the following statements: p. 3, "If a small amount of reticular tissue is boiled with a large quantity of water for 24 hours it is dissolved and turns into an opalescent fluid, from which a voluminous precipitate will be precipitated by acetic

[1] This *Journal*, xxvii. p. 463.

[2] M. Siegfried, *loc. cit.*

acid," and p. 19, "If reticulin is boiled continuously with water, it forms a slightly opalescent fluid. One gram tissue was boiled uninterruptedly for 36 hours in four litres of water. Only a very small residue remained undissolved on the filter." Miss Tebb was therefore not justified in thinking her residue to be different from reticulin.

In her second experiment, Miss Tebb again did not follow my method. She extracts the mucosa of a dog with a 1 % solution of sodium carbonate, then digests with trypsin. The tissue was then washed successively with water and alcohol, and then kept under ether for 23 days. After that, it was boiled for 8 hours at intervals of 20 to 80 minutes. The tissue remained unchanged in appearance after this process, and the extracts yielded no jelly. After the tissue was boiled over-night, there was a small residue. Among other reactions, the solution also gave a precipitate with acetic acid.

In this experiment, therefore, the decomposition of the reticular tissue and formation of reticulin, did not occur as rapidly as I have noticed it. Miss Tebb looks at the prolonged treatment with ether as the cause. She then makes an experiment with undigested mucosa (cat) and considers her opinion confirmed.

I had split up 25 gr. reticulin with hydrochloric acid and tin dichloride, and after eliminating the bases with phosphotungstic acid I did not see any separation of glutaminic hydrochloride from the hydrochloric syrup.

From this I concluded, p. 14, that, as glutaminic hydrochloride is almost insoluble in ice-cold concentrated hydrochloric acid, there is either none or very little of this amido acid formed by the decomposition of reticulin. Miss Tebb believes that reticulin is collagen, which has been changed by alcohol and ether. She made experiments to obtain glutaminic acid by splitting up reticular tissue which had been boiled out. The mucosa of 9 cats was digested, treated with alcohol for 2 hours, and then kept under ether for 7 days. She obtained 0·27 gr. dry tissue, which she boiled in 50 c.c. of water four times, and each time for half-an-hour. Each extract yielded a jelly. The remainder, the weight of which was 0·27 gr. minus the weight of the gelatin which was in the four extracts (a quantity sufficient to yield a jelly each time), was decomposed by hydrochloric acid and tin bichloride. The tin was removed by sulphuretted hydrogen, the filtrate concentrated and then saturated with hydrochloric acid gas. At first, cubical crystals of an inorganic nature were deposited (probably sodium chloride), but after cooling the fluid with ice and salt, a considerable

amount of small needles was deposited. As the quantity, nevertheless, was too small to undergo an analysis, the crystals prepared from gelatine under the same conditions were used to determine the percentage of nitrogen. This turned out to be the same as that calculated to be in glutaminic hydrochloride.

In my opinion this is no proof of the formation of glutaminic acid from reticular tissue that has been boiled out. With small quantities like that it cannot be proved. Even if the formation of glutaminic acid was proved in this experiment, it would not conflict with what I found, namely, that *reticulin* forms none or very little glutaminic acid when decomposed by hydrochloric acid and tin bichloride. What Miss Tebb decomposed was not reticulin, but reticular tissue which had not been sufficiently boiled and split up.

Although Miss Tebb did not follow my method in her experiments, nevertheless, to judge from my work with reticular tissue that had been but once digested, her tissue ought to have more rapidly split up into reticulin and gelatin. It is true that these experiments again differ from mine, in that she treated the tissue with a 1 % solution of sodium carbonate before it was digested.

For this reason I prepared the intestinal mucosa of 3 pigs and made a study of the decomposition of the tissue by treating it in different ways.

Exp. I. One part was treated as before, viz. the fat extracted, digested, the fat again extracted and again digested. The tissue was then boiled in a reflux-condenser and the filtrate of the partially decomposed tissue concentrated. The result was a firm jelly. After entirely drying at a temperature of 110° C. this gelatin weighed 0·1709 gr. The residue was boiled with fresh water for 20 min. The filtrate gave only a slight precipitate with tannin. After it was entirely decomposed, the precipitation of reticulin was hastened by addition of ammonium acetate. The reticulin was put on a weighed filter and dried at 110° C. The weight was 0·2772 gr.

The 20 min. boiling, therefore, split off 0·1709 gr. gelatin from a reticular tissue containing 0·2772 gr. reticulin. Although the tissue was uninterruptedly extracted with alcohol and ether in a Soxhlet's apparatus, it yielded the greater part of the gelatin within 20 min.

This contradicts Miss Tebb's opinion, that the collagen of intestinal mucosa gives up the property of rapidly being transformed into gelatin by boiling with water through the influence of alcohol and ether upon it.

Here may be mentioned, that after I had noticed the rapid formation of gelatin from reticular tissue, Mörner[1] made the same observations in regard to fish-scales.

In several experiments I noticed that some preparations decomposed more slowly than others. In these cases, the fluid proved to be alkaline, although the tissue had been carefully washed beforehand, until it gave a neutral reaction. In the event of the reticular tissue giving an alkaline reaction after boiling for ½ hour, a few drops of hydrochloric acid added to the boiling fluid in a short time entirely decomposed the tissue. The reticulin floats then as a fine powder in the fluid and gradually deposits. This action is hastened on addition of sodium chloride or, as has already been stated, of ammonium acetate.

I think this is the reason why Miss Tebb did not see a decomposition even after boiling for hours, namely, that she treated the tissue with a 1 % solution of sodium carbonate before beginning her experiment.

The use of very dilute hydrochloric acid instead of water has the advantage of hastening the process of decomposition of the reticular tissue and the splitting off of reticulin, and also reducing the quantity of water necessary. The following experiments were made with very dilute hydrochloric acid.

Exp. II. Intestinal mucous membrane (pig) that had been washed, digested, washed, the fat extracted, and again digested and washed was put into 80 c.c. of water. To this was added 20 c.c. of 0·25 % hydrochloric acid, making the percentage of acid in the fluid equal to 0·05 %. This was boiled and the tissue was entirely decomposed in 20 min. The reticulin was separated into little granules and was gradually deposited. The addition of sodium chloride or ammonium acetate was found to favour the precipitation.

Exp. III. Intestinal mucosa (pig) that had been washed, digested, washed again, but the fat not extracted, was put into 80 c.c. of water with the addition of 20 c.c. of 0·25 % hydrochloric acid; 20 minutes boiling decomposed the tissue. The reticulin separated in little granules, and was gradually deposited. The addition of sodium chloride or ammonium acetate favoured the precipitation.

This experiment excludes the possibility suggested by Miss Tebb that reticulin is collagen which has been changed by alcohol and ether, and it verifies my former statements.

[1] *Zeitschrift f. physiol. Chemie*, xxiv. p. 125.

EXP. IV. Intestinal mucosa that had been thoroughly washed and extracted for 24 hours with a 1 % solution of sodium carbonate, and carefully and thoroughly washed as above, was boiled with a 0·05 % solution of hydrochloric acid. Even after 2 hours the decomposition of the tissue was not complete.

In all these experiments I used practically the same amount of mucosa.

These experiments show the ready decomposition of digested reticular tissue when boiled with a 0·05 % solution of hydrochloric acid.

CONCLUSIONS.

1. The work of Miss Tebb does not invalidate the fact that reticular tissue of intestinal mucosa consists of collagen and reticulin.

2. Reticular tissue which has been treated according to my directions decomposes more rapidly when boiled with a 0·05 % solution of hydrochloric acid than when boiled with water.