CONTRIBUTIONS TO PHYSIOLOGICAL
AND
PATHOLOGICAL CHEMISTRY

(1) On the Chemistry of the Thyroid Gland and the Nature of its active ingredient.
(2) On the Chloride excretion in Pneumonia.

Being a Thesis submitted to the UNIVERSITY OF EDINBURGH FOR THE DEGREE OF DOCTOR OF MEDICINE by ROBERT HUTCHISON, M.B., C.M., (1893) M.R.C.P.E.

April 1896.
This Thesis consists of two parts. These have no connection with one another other than this, that together they represent the chief part of the work which the writer has done in the domain of physiological chemistry since his graduation as a Bachelor of Medicine in 1893. Neither part, however, is purely chemical; both will be found to have very direct bearings on clinical Medicine. The first part deals with the chemistry of the Thyroid gland and the nature of its active constituent. It was in great measure at the suggestion of Professor Greenfield that I was induced to make a special study of this subject, but in part also, from the recognition of the pressing need for its investigation from the chemical and clinical sides simultaneously. As the result of my work I have been able to come to some positive conclusions as to the nature of the active ingredient of the Thyroid gland which will be found, I think, to throw fresh light on the physiology and pathology of that organ.

The chemical work in this subject was carried out entirely in the Physiological Laboratory of this University during the time that I have had the honour
of acting as Assistant to Professor Rutherford. The clinical observations were made as opportunity afforded, partly in the wards of the Royal Infirmary, partly in the Deaconess Hospital.

In the second part of the Thesis, the chloride excretion in Pneumonia is considered. This is in no sense a new subject. Indeed, it is now more than a generation ago since it attracted considerable attention. The writer's interest in it was first excited by observing the striking behaviour of the urinary chlorides in some cases of pneumonia in children. The problem presented itself, what is the cause of the disappearance of the chlorides from the urine in pneumonia? All the replies to this question, which had hitherto been suggested, appeared to be based on insufficient evidence and required to be tested. Before proceeding to this, however, it seemed necessary to focus more definitely the clinical facts. Accordingly the first section of this part of the thesis deals with the clinical aspects of the question. The work in connection with it was carried out in the University Medical Wards of the Royal Infirmary during the writer's tenure of the Stark Scholarship. In the second section the theories hitherto advanced to
account for the clinical phenomena are subjected to a systematic examination in the light of analytical and experimental evidence. They are all found more or less wanting. The investigation is then pushed further, fresh facts being brought forward. It will be found, however, that these do not warrant any dogmatic statement and the conclusion it arrived at that no thoroughly satisfactory explanation of the clinical phenomena can as yet be given. This negative result is somewhat disappointing but the writer was not unprepared to meet with it. His work, at all events, has cleared the ground for future observations and has scrutinised all previously known facts besides advancing some that are new. It only remains to be said that the analytical and experimental work in the second section was carried out in part at the Institute for Physiological chemistry at Strassburg and in part at the Physiological Laboratory of this University.

With these words of introduction I would humbly submit this Thesis to the University in the hope that it may be found not unworthy of its approval.
PART I

ON THE CHEMISTRY OF THE

THYROID GLAND

and

THE NATURE OF ITS ACTIVE INGREDIENT.
I. CHEMICAL.

When one thinks of the immense amount of research which has recently been devoted to the Thyroid gland and of the number of papers which have appeared dealing with its histology, normal as well as morbid, and with its clinical and therapeutic aspects, one is surprised that so little attention, in comparison, has been bestowed upon its chemistry. And yet if we are ever to have a clear notion of the nature of the functions which the thyroid performs in the healthy body and of the rôle which it plays in pathological processes, it is to Chemistry that we must look for light. For it is Chemistry alone that can solve for us the central problem which clinical observation has raised: — What is the active substance produced by the thyroid gland?

In what follows I propose to deal first with the chemistry of the gland and then to proceed, in the light of the results which it affords, to attempt a reply to the question which has just been stated.

As has already been indicated the chemical literature of the thyroid is comparatively limited in extent. In place, however, of giving a summary of
what is already known it will be more convenient to refer to the work of others under each point as it is raised. I may, therefore, proceed at once to give the results of my own observations.

In investigating the chemistry of the thyroid, I have used exclusively the sheep's gland. These were obtained as fresh as possible from the butcher and carefully freed from all adherent connective tissue and fat as a preliminary to all further investigations. The gland consists of two separate lobes. The weight of each of these is, on the average, about $2\frac{1}{2}$ - 3 grammes or about 45 grains. This standard we shall have frequent occasion to refer to in what follows; it applies of course to the fresh or undried gland.

1. Proteids of the Thyroid.

A thesis dealing with this subject was submitted to this University by Gourlay in 1893 and was subsequently published in the sixth volume of the Journal of Physiology. Gourlay states that the only Proteid that can be obtained in any quantity from the thyroid is a nucleo-albumin. I started my observations, therefore, by attempting to isolate this body. Like Gourlay, I employed Halliburton's method. That
is to say the fresh thyroids were chopped up and pounded with about their own bulk of common salt in a mortar. The resulting viscous mass was poured into excess of distilled water and rapidly stirred. By this method any nucleo-albumin present floats to the top, globulins, if the excess of water be sufficiently great, fall to the bottom along with the debris of the glands, while other proteids remain in solution. In the case of the thyroid I have found the yield of nucleo-albumin to be very small. In this my results do not at all agree with those of Gourlay. Comparing for example, equal weights of fresh thyroid and fresh thymus, almost the entire amount of proteid present rises to the top in the case of the latter, while in the case of the thyroid, only a thin layer collects upon standing. This layer was removed and dissolved in 1% sodium carbonate solution. It was then freed from particles of fat by filtration and the nucleo-albumin precipitated from its alkaline solution by acetic acid. The precipitate was collected, washed and dried. Even using as much as 60 grammes of fresh thyroid, I have been unable to obtain more than a few centigrammes of dried nucleo-albumin. The latter when digested with pepsin and hydrochloric acid left a

Nucleo-
Albumin of the Thyroid.
relatively large residue. This was found to be rich in phosphorus, but I have not been able to collect enough to make it worth while to examine the residue for nuclein bases. I think there can be no doubt, however, that the substance is really a nucleo-albumin. Gourlay has found - further, that it corresponds with other nucleo-albumins in producing intravascular coagulation of the blood. I think it very probable that the nucleo-albumin is derived from the cells of the thyroid gland or from their nuclei, but I am sure that Gourlay was in error in regarding it as the principal proteid furnished by the gland. The reason for this opinion will now be set forth.

As long ago as 1884 Bubnow published in the Zeitschrift f. phys. Chemie (Band VIII) a paper on the chemistry of the thyroid of the calf in which he described certain proteids which he had separated from the gland and to which he gave the name of Thyreo-protins. He got these by extracting the glands with saline or alkaline solutions and then adding acetic acid. The "thyreo-protin" was precipitated. I tried if a similar substance could be obtained from the thyroid of the sheep. Having skimmed off the layer of nucleo-albumin which, as has been described,
rises to the top in Halliburton's method of preparation, I decanted the subjacent watery solution from off the fragments of tissue which had fallen to the bottom of the vessel. On adding a few drops of 2% acetic acid to the fluid a very abundant precipitate appeared. The watery solution, therefore, contained, even after removal of all nucleo-albumin, a proteid substance in large amount. This was found to present all the characters of the Thyreoprotin precipitated by Bubnow from an extract of thyroids made with 10% salt solution and recognised by him as identical with what has long been known to histologists as the 'colloid' matter of the thyroid. This is unquestionably the chief proteid, or proteid-like substance to be obtained from the thyroid. Sixty grammes of fresh thyroid yield on an average about six grammes of dried colloid - a proportion of about 1 in 10 while we saw that a similar quantity of the gland yielded, by Halliburton's method, only a few centigrams of dried nucleo-albumin.

I propose, throughout this paper, to refer to this substance always as 'colloid' because I regard it as undoubtedly the substance known to the minute anatomists of the thyroid under that name. The additional
proofs of this identity which are furnished by microchemistry must be deferred till later. It remained, now, to prepare this substance in a state of greater purity. For this purpose, extracts of thyroids were made with various solutions. I have tried for this purpose the use of distilled water, of 5% magnesium sulphate, 10% common salt, 1% sodium phosphate and very dilute (0.1% or so) caustic soda solutions. Whilst the colloid matter is extracted by all of these it is so rather unequally. Much the largest yield is furnished by a dilute alkaline solution - preferably the above solution of caustic soda. The extract was in every case made by chopping the fresh glands very small and placing them in a flask containing the solution to which a few drops of chloroform were added to prevent putrefaction. The flask and its contents were then thoroughly shaken and left standing at the temperature of the room for 24 hours. The fluid was then strained through muslin and filtered to remove any particle of fat. The remains of the gland were then returned to the flask and re-extracted as before. The filtered extract is of a reddish colour, and, where an alkaline solution was employed, of a somewhat ropy consistency. It was poured into tall jars, a
few drops of 2% acetic acid added and the whole stirred. The fluid immediately became opaque and very shortly flocculi appeared which, on standing over night, settled to the bottom as a thick layer of a pale brownish white colour leaving a clear reddish supernatant fluid in which acetic acid produced no further precipitate. It should be mentioned that the addition of acetic acid requires care. If one adds the least excess the fluid simply becomes opaque - no flocculi separating even on long standing. Further than this, the colloid is much more easily precipitated from some solutions than from others. It comes down most readily out of slightly alkaline fluids - fairly easily out of salt solution - not very easily out of 5% Magnesium sulphate. The precipitate was next separated by decantation and re-dissolved in distilled water and a few drops of caustic soda. From this solution it was again precipitated by acetic acid and this re-solution and precipitation was repeated usually at least twice or three times in order to ensure as great purity as possible.

I find the pure fresh colloid as above prepared to have the following characters which, for the sake of clearness, will be taken up under certain heads.
Solubilities: It is soluble, although slowly, in distilled water, more readily in dilute saline solutions, and extremely readily in weak solutions of alkalies — even of such a feeble alkali as phosphate of soda \( \text{Na}_2 \text{HPO}_4 \). From all its solutions it is precipitated by the addition of an acid, although as stated above, more readily from some solutions than from others. The precipitate is soluble in excess of acid, even of acetic acid, thus differing from mucin.

In addition to acids it is precipitated from its solutions by saturation with sulphate of ammonia or sulphate of magnesium. It is also precipitated by saturation with common salt, but more readily from alkaline than from saline solutions.

Effect of heat: This varies very greatly with the nature, and especially with the reaction, of the solution. It is not coagulated even by boiling in an alkaline solution. It is coagulated by boiling in a neutral solution of magnesium sulphate (5%). On boiling a 10% salt solution, the fluid becomes very opalescent but no true coagulum forms.

I then tried to determine the fractional heat coagulation point in the usual way. A solution in
5% magnesium sulphate was tried, the solution being rendered very slightly acid with acetic acid. The fluid became very opalescent a little below 50°C. and at 57°C. flocculi separated out in abundance. The solution was kept at this temperature for five minutes and then filtered. On adding alcohol to the filtrate no further precipitate appeared. The whole of the substance had therefore come down at 57°C. and this is to be regarded as the point of fractional heat coagulation of the colloid in 5% magnesium sulphate solution. Bubnow gives precisely the same temperature for his 'Thyreoprotin'. It must be noted, however, that the temperature of coagulation of the colloid varies very much with the reaction of the fluid and also, apparently, with the amount of saline matter present in the solution. For example, if one takes the watery solution obtained after removing nucleoalbumin by Halliburton's method and adds to it a drop or two of very dilute acetic acid till slight opalescence has just appeared and then slowly heats the fluid, it will be found that the colloid coagulates at a very low temperature - 35° - 40°C. The tendency for it to separate out of an acid solution appears to be so great that it is in such circumstances at once
thrown down by even a very moderate degree of heat. That this result is not to be attributed to the presence of a large amount of salt is shown by the fact that the same result was obtained after removal of most of the salt by dialysis.

(3) **Reactions.**

The ordinary proteid reactions were tried. All gave a positive result. The precipitate given by nitric acid was very readily soluble in excess.

(4) **Effect of Hydration.**

A considerable quantity of the fresh substance was boiled for two hours in 5% sulphuric acid with an inverted condenser. There resulted a dark brown fluid and a small amount of a finely flocculent brownish deposit. This deposit will be referred to later. Part of the fluid was neutralised with caustic potash. The sulphate of copper test gave a brilliant biuret reaction but showed no sign of the presence of any reducing substance. The colloid, therefore, yields on hydration albumoses and peptone but no cupric oxide reducing body, which indicates that it is not allied to mucin. Another part of the acid solution was neutralised with baryta water, the
precipitate of barium sulphate filtered off, the excess of barium removed by a stream of carbonic acid gas and the filtrate rendered alkaline with ammonia; the precipitate of phosphates was then removed and nitrate of silver solution added to the clear filtrate. Not a trace of a precipitate resulted. One can, therefore, conclude that the substance yields no nuclein bases on boiling with sulphuric acid, and can infer from that that it contains no true nuclein.

(5) **Elementary Analysis of the 'Colloid'.**

Bubnow, in the paper already referred to gives the results of the elementary analysis of his 'Thyreoprotin' obtained from the thyroid of the calf. The following is his table of the average percentage composition:-

<table>
<thead>
<tr>
<th>Element</th>
<th>Percentage</th>
</tr>
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<tbody>
<tr>
<td>C</td>
<td>49.36%</td>
</tr>
<tr>
<td>H</td>
<td>6.45%</td>
</tr>
<tr>
<td>N</td>
<td>16.04%</td>
</tr>
<tr>
<td>S</td>
<td>1.38%</td>
</tr>
<tr>
<td>O</td>
<td>28.77%</td>
</tr>
</tbody>
</table>

He makes no statement regarding the ash constituents.

Before I made any analyses of the colloid it was purified in the following way. Having been separated in the manner already detailed and thrice
re-precipitated by acetic acid, it was washed with alcohol and then placed in fresh alcohol at 40°C. for 24 hours. The alcohol was removed by filtration and the substance thoroughly washed with ether. The final alcohol and the ether washings were found, on application of the cholin test, to be free from lecithin. The substance was then dried to constant weight at 105°C. There resulted a very fine pale brown powder devoid of taste or of odour. It was found impossible, even by repeated washing with hot and cold ether, to render the substance colourless. I estimated in a small quantity of it (0.8 grams) the amount of nitrogen and of phosphorus, these being regarded as the two most important substances to determine. Weibull's method (oxidation by means of strong sulphuric acid and sulphate of copper) was the method employed. The nitrogen was determined as in Kjeldahl's process. It amounted to 15.5%. (This, it will be observed, is very close to the amount estimated by Bubnow in his Thyreo-protein - 18%). The phosphoric acid was precipitated by magnesia mixture from the acid solution previously neutralised with ammonia and the magnesium pyro-phosphate washed, incinerated and weighed in the usual way. The result
shewed the presence of a very small amount of phosphorus = 0.045%. Such a small amount might, of course, be due to the presence of impurities, especially to lecithin or adherent calcium phosphate. The former, however, was apparently all removed by the alcohol and ether and the presence of the latter was unlikely after the repeated precipitations of the substance with acetic acid, so that one is, I think, entitled to conclude that the colloid matter contains phosphorus although in very small amount.

This is in harmony with what was found by Gourlay - that the colloid matter in the alveoli of the thyroid gives a positive result with the method of Lilienfeld and Monti for the microscopic localisation of phosphorus. Its very small percentage amount, however, practically excludes the colloid from the group of nucleo-albumins. I now come to one of the most interesting points in the chemistry of the colloid matter - the composition of its ash.

About \( \frac{1}{2} \) a gramme of the dried colloid matter was taken, and to it was added about 1 gramme of pure caustic soda dissolved in a very small quantity of water and in addition a few chrystals of pure potassium nitrate. The mixture was then carefully
evaporated to dryness in a porcelain capsule and gently heated, avoiding as far as possible even the appearance of dull redness, till a colourless mass was left. This was dissolved in a little distilled water and filtered. To the filtrate a little nitric (containing nitrous) acid was added. A pale brown solution resulted. On shaking this up with a small quantity of chloroform the latter assumed a brilliant purplish red colour. This indicated the presence of iodine. A few drops of sulphurous acid were then added and then some starch solution. The latter at once assumed a deep blue colour. The presence of iodine was thus confirmed. (It need scarcely be said that all the re-agents employed were examined and found to be iodine free.) The fact of the existence of iodine in the colloid matter of the thyroid is very striking. Baumann in a paper published in the Zeitschrift f. phys. Chemie (Bd. XXI, Heft 4, December 28, 1895) and entitled 'über das normale Vorkommen von Jod im Thierkörper' has described an organic compound containing iodine which he has extracted from the thyroid. His method consisted in boiling the entire gland with 10% sulphuric acid. An amorphous residue is left from which the iodine containing body is separated by

Colloid contains iodine.
boiling with alcohol. He has named the substance 'Thyroiodin.' I have examined the amorphous substance which is left behind on boiling the colloid matter with sulphuric acid (vide 'Hydration of the Colloid') and I find it to be relatively much richer in iodine than the colloid itself. I have little doubt that it must be identical with the amorphous substance obtained by Baumann from the entire gland. Of the actual amount of iodine present in the colloid matter I shall speak later. The qualitative determination of the other ingredients of the ash was made by heating some of the dried colloid in a platinum crucible till all fumes had ceased to come off and a black friable mass was left. This was extracted first with hot water and subsequently with dilute hydro-chloric acid. In this way a watery and an acid extract were obtained. The residue was dried and ashed with the filter paper - practically nothing was left. The watery extract gave only a faint iodine reaction on acidifying some and shaking up with chloroform. Nitrate of silver produced merely an opalescence - unaffected either by nitric acid or by ammonia. Probably most of the iodine had been driven off owing to the absence of a base to fix it. Ammonium molybdate and nitric acid
gave a distinct yellow precipitate indicating the presence of phosphorus. Nothing else could be detected in the watery extract. In the acid extract no calcium could be detected and only a small amount of sulphuric acid.

The most important facts which we have learned from the analysis of the colloid are that it contains about $15\%$ of nitrogen, a low percentage of phosphorus (0.04) and a small amount of iodine. We may now pass to -

(6) Effects of gastric digestion upon the colloid.

In these experiments the fresh colloid was obtained in the usual way; after washing with ether it was placed in about twice its volume of $0.25\%$ hydrochloric acid and left for a few hours at $40^\circ C$. At the end of this time all traces of ether had disappeared and the substance had swelled up into a jelly. Pepsin hydrochloric acid solution (made by extracting the mucous membrane of the pig’s stomach with $0.25\%$ hydrochloric acid) was then added and the mixture placed in the incubator. Liquefaction of the colloid resulted very rapidly. In about half an hour the substance was reduced to a dark brown fluid. At the end of twenty hours nothing had separated out and the fluid showed the presence of albumoses and peptone. At the end
of three days a small amount of an amorphous brownish substance was deposited. To this substance we shall return immediately. Having determined these general features of the gastric digestion of the colloid, viz. that it is very rapidly liquefied, that this liquid early shows the presence of hydrated proteid, and that it is only after somewhat prolonged digestion that any residue separates out, I proceeded as follows.

Some fresh colloid was digested for 20 hours. The fluid was then filtered, no residue being left. The filtrate was then saturated with sulphate of ammonium. A considerable amount of material separated out and floated to the top of the saturated fluid. This material was removed to a beaker and distilled water added. A large part of the substance dissolved. A considerable residue, however, was left and settled to the bottom in a flocculent form. It was collected on a filter and was found to dissolve very readily in dilute alkali. From its solution it was easily precipitated by the addition of acetic acid in the form of brownish-grey flocculi. These were collected on a filter, re-dissolved in alkali and the solution examined. It was found on application of the sulphate of copper test not to give even a trace of a violet
colour. The substance was then precipitated by acetic acid, re-dissolved in excess and a drop of ferrocyanide of potash solution added. There was no precipitate. The substance, therefore, is proteid free. We are confronted, that is to say, with the remarkable fact that if one submits the colloid matter to gastric digestion and saturates the resulting fluid with sulphate of ammonia, there is precipitated along with the albumoses, a substance which differs from the latter in being not dissolved again in the addition of water and in the fact that it is entirely proteid free.

I next attempted to isolate this remarkable substance from the products of digestion in another way than by precipitating it along with the albumoses. I found that on the cautious addition of dilute caustic soda to the filtered digestion, a point was reached at which, although the fluid was still slightly acid, a flocculent precipitate fell out. This was collected, dissolved in dilute alkali and re-precipitated by acetic acid. It was found on examining the substance in alkaline solution that it also was proteid free. It was, therefore, probably identical with the substance already obtained by saturation with sulphate of ammonia. To finally determine whether it
was really identical or not one had only to proceed as follows. The filtered product of digestion was almost neutralised with caustic soda. The flocculent precipitate removed and the filtrate saturated with sulphate of ammonium. The precipitate was separated and water added to it. It dissolved completely. In other words it contained none of the proteid-free body the latter having been entirely removed by the previous addition of alkali. The two substances are, therefore, identical.

We may now return again to the substance which separates out spontaneously on prolonged digestion. Some of this substance, resulting from three days digestion, was collected, washed with dilute acetic acid, and dissolved in dilute alkali. The solution was proteid free. It therefore resembles the substance already obtained. The further proof of its identity was furnished as before by removing the substance after it had fallen out and saturating the remaining fluid with ammonium sulphate. On solution of the precipitate no residue was left, i.e. no proteid-free body was remaining in the solution.

One may summarise the result of these experiments in this fashion.- By the gastric digestion of
the colloid matter, the latter is split up. There results from this splitting (1) hydrated proteid = albumoses and peptone. (2) an amorphous substance which has the peculiarity of being entirely proteid free. The latter separates out spontaneously if the digestion be prolonged. It can be obtained, however, even at an early period of digestion in one of two ways, either by adding to the digestion dilute alkali just short of neutralisation when the proteid free body is precipitated or by saturating the solution with sulphate of ammonium, in which case the proteid free body is precipitated along with the albumoses and can be separated from the latter by re-dissolving the precipitate in water. The albumoses go into solution and the proteid free body remains as an insoluble residue.
Further examination of the proteid-free body.

The substance was prepared by peptic digestion of the colloid in the manner described. It was purified by reprecipitation from its alkaline solution by means of acetic acid and was then washed with alcohol and finally with ether. It was then dried to constant weight at 110°C. A dark brown powder resulted without taste or odour. It was practically insoluble in water but was readily dissolved in alkali even if very dilute. The solution was entirely free from any trace of the presence of proteid matter. I have only been able to prepare a little more than one gramme of the substance in all. This represents rather more than 200 grammes of fresh thyroid. In the substance so prepared I have estimated the percentage of nitrogen, of phosphorus and of iodine. The two former were done by Weibull’s method. The nitrogen amounted to 12.9%. The phosphorus was precipitated by molybdate of ammonium the precipitate washed with this solution, dissolved in ammonia and reprecipitated by magnesia mixture. The magnesium pyrophosphate was collected and weighed after incineration in the usual way. It represented an amount of phosphorus...
equivalent to nearly 0.9%. The iodine was estimated as follows:— Half a gramme of the substance was taken and mixed with one gramme of pure caustic soda which had been dissolved in a minimum of water. The mixture was slowly evaporated to dryness and carefully incinerated in a porcelain capsule with the aid of a small quantity of potassium nitrate at a temperature below even dull redness. The almost colourless ash was allowed to cool, dissolved in water and filtered. It was then rendered acid with strong nitric acid. The solution became brown (= liberation of iodine). A few drops of sulphurous acid were then added to reduce all the iodine present to the form of hydr-iodic acid. Solution of nitrate of silver was then added in excess. A pale yellow precipitate of iodide of silver at once appeared and was allowed to settle to the bottom. It was then collected on an ash-free filter, washed with dilute nitric acid and then with ammonia, dried, incinerated, weighed, and the amount of iodine calculated from the iodide of silver found. It amounted to not more than 0.7%. We may compare the amount of nitrogen and of phosphorus in the original colloid and in the proteid-free substance.
thus.-

<table>
<thead>
<tr>
<th></th>
<th>Colloid</th>
<th>Proteid-free substance</th>
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<tbody>
<tr>
<td>N.</td>
<td>15.5%</td>
<td>12.9%</td>
</tr>
<tr>
<td>P.</td>
<td>0.04%</td>
<td>0.9%</td>
</tr>
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The proteid-free substance is thus seen to be poorer in nitrogen than the mother substance but considerably richer than the latter in phosphorus. Now as the amount of the proteid-free substance obtainable from a given amount of colloid is about $\frac{20}{29}$ of the weight of the latter, it would seem as if all the phosphorus in the colloid really belongs to the proteid-free part of the latter. Is the same true of all the iodine in the colloid?

Now, I have not been yet able to estimate the amount of iodine in the original colloid owing to the large amount of the latter which would be required to give an accurate result, but one can arrive at some sort of idea on the subject in another way. Is any iodine split off with the albumoses during digestion of the colloid? The reply is in the affirmative. I have always been able to obtain distinct evidence of the presence of iodine in the albumoses obtained on peptic digestion of the colloid, even when the...
digestive process was only allowed to go on for a comparatively short time (1½ hours) One would infer from this that the iodine in the colloid stands in relation both to its proteid and to its non-proteid part. In as much, however, as the albumoses always appear to contain relatively less iodine than the proteid-free residue, the union of the iodine with the non-proteid part of the colloid must be more intimate than with the rest of it, and on hydration of the colloid, the splitting off of the iodine from its non-proteid part seems only to occur with difficulty. On the exact nature of the non-proteid part of the colloid it would be premature to pronounce an opinion until one had made a fuller examination of it. I have only been able to ascertain some of its general characters. I have stated that it gives no trace of a colour with the sulphate of copper test, and that it does not give a precipitate with ferrocyanide of potash. I may add that with Millon's re-agent, it gives a precipitate which on heating becomes only of a dull red colour; it gives no precipitate with solution of potassio-mercuric iodide. It is precipitated by mercuric chloride and by phosphotungstic acid in the presence of excess of hydrochloric acid but the precipitate is
not abundant in either case. It does not yield an acid reaction nor does it precipitate proteids from their solutions.

I have made an experiment to see whether the same substance is obtained on pancreatic digestion of the colloid. A quantity of the latter was dissolved in 1% sodium carbonate solution and some Liquor Pancreaticus added. The solution was left in the incubator over night. Next morning it was rendered slightly acid by the addition of 0.25% hydrochloric acid. A flocculent precipitate fell out in considerable abundance. This was found to be readily soluble again in dilute alkali and the solution was proteid-free. On examining the substance for iodine, however, it was found that it apparently did not contain the latter in nearly so large amount as the proteid-free body obtained on peptic digestion. On the other hand the albumoses produced in tryptic digestion apparently contained a larger quantity of iodine than those which resulted from the action of pepsin. One would conclude, therefore, that the more powerful hydrolytic action of trypsin had caused a larger proportion of the iodine to be split off with the proteid
part of the colloid.

I must now say a word on the relations of this body to Baumann's Thyroiodin. As already mentioned, Baumann has extracted from the thyroid an iodine containing substance to which he has given the above name. His method of getting it was as follows. The thyroids were boiled for several hours with 10% sulphuric acid. An amorphous residue was left. This he extracted with boiling rectified spirit and on evaporation of the spirit obtained a brownish substance which is his thyroiodin. It apparently resembles in many points the substance I have described. It is readily soluble in alkalies and the solution is proteid-free. It contains, however, only about half as much phosphorus as the substance obtained by digestion. This is not surprising as the prolonged treatment with sulphuric acid is bound to have removed some of the phosphorus. On the other hand it contains about twice as much iodine as the other substance - or rather more than 2%. This is rather surprising as some of the iodine must, to judge at least by what happens on digestion - have gone off with the albumoses formed by hydration. It is possible, however, that the amorphous substance obtained after boiling with the
acid and before treatment with spirit is relatively poorer in iodine than the substance ultimately obtained and that it is really split up on boiling with alcohol - the part which contains the iodine alone going into solution. If this be so the residue after boiling with acid would more nearly correspond to the substance I have myself described than does the Thyro-iodin itself. Unfortunately Baumann gives no estimate of the amount of nitrogen in his substance so this aid to identification is lost.

On the whole I should be inclined to regard Thyro-iodin as an artificial product, derived from the colloid by the treatment to which it has been subjected. On the other hand, the substance I have myself described is obtainable in a much simpler and more natural way - by simple gastric digestion of the colloid matter. I consider, therefore, that its study is more likely to throw light on the composition of the colloid as it exists in the living body than is that of the substance described by Baumann.

I have now described in detail two proteid bodies which can be obtained from the thyroid and the question arises - Does the thyroid contain any other proteid? To this question I can reply briefly for my
results as far as it is concerned are fully confirmatory of those already published by Gourlay. The latter has shown that the fresh thyroid yields no proteases or peptone. As regards proteids, other than the two already described, the presence of which can be shown in thyroid extracts, I agree with Gourlay in regarding them as derived from the blood and lymph necessarily contained in the gland. I find present in watery and saline extracts of the gland after removal of the colloid matter a small amount of proteid which coagulates at 75°C. This is the coagulation point of serum albumin. I find also, as Gourlay has pointed out, that the more free from blood and lymph the thyroid is rendered, the less is the quantity of this substance obtained. It seems reasonable to regard it as serum albumin. I have not been able to discover the presence of any other proteid, after the removal of those already described the extract is proteid-free.

We may now pass to a consideration of the substances present in this proteid free extract, speaking of them under the general term 'Extractives'.

Bubnow, in the paper already referred to, showed that he had been able to demonstrate the presence in
the thyroid of creatin, xanthin, hypo-xanthin and sarcolactacid acid. These are such extractives as might be expected to be found in almost any organic tissue. Recently Fraenkel (1) has stated that he has discovered in the thyroid a crystalline substance to which he has given the name Thyreo-antitoxin. He obtained this body by evaporating a proteid-free watery extract to a syrup, and then taking up the syrup with alcohol. On adding ether to the alcoholic solution, the substance crystallised out. The crystals are very hygroscopic and their solution gives the general reactions of alkaloids. He has made an analysis of the substance and calculates for it the formula

\[ C_6 H_4 N_2 O_2 \]

Even more recently a paper has appeared by Drechsel-(2) in which he describes a method by which he has obtained from watery extracts of the thyroid of the pig, after removal of all proteids, two bodies, one of which he believes to be identical with the substance described by Fraenkel. My own work on the nature of the extractives of the thyroid has no pretence to being exhaustive. So far as it goes, however, it would simply confirm that of Bubnow. In watery extracts of the thyroid I have only been able
to discover such extractives as are usually met with - creatin, xanthin and hypo-xanthin. I have attempted to obtain Fraenkel's body by evaporating the watery extract of 65 grammes of fresh thyroid to a syrup and then extracting the latter with absolute alcohol, concentrating the alcoholic solution and adding to it ether in excess. No crystalline substance separated out. Further, on making an extract in a similar way with an equal quantity of fresh thymus, I could detect no difference in the extractives present in the two solutions, both gave the same reactions. I admit however, that the complete investigation and identification of the amido bodies which may be met with in organic extracts is an extremely difficult matter requiring much more experience in organic chemistry than I possess, and until a more detailed description of these bodies which are alleged to exist is published, I would prefer to leave it an open question whether the thyroid does or does not yield extractives which are peculiar to that gland. I would only add that I have been unable to discover the presence of any iodine holding substance in the watery extract after removal of all proteid matter.
I may summarise the conclusions at which I have arrived as the result of the chemical examination of the thyroid as follows.

1. The Thyroid contains two proteids:
   
   (a) A nucleo-albumin, present in very small amount and resembling in its characters the nucleo-albumins obtainable from other cellular organs. It is probably derived from the cells lining the acini of the thyroid.
   
   (b) A substance which may be described as the 'colloid' matter. This is present in large amount. It may be provisionally regarded as a compound proteid which contains, in addition to the elements present in other proteids, phosphorus and iodine. It can be readily split up by hydration into a proteid (\textit{nucleo-albumin}) and a non-proteid part. It is probable that all the phosphorus and most of the iodine belongs to the non-proteid part. There is no evidence that this substance contains either nuclein or para-nuclein.

2. The Thyroid contains the ordinary extractives - creatin, xanthin etc. It has recently been
asserted to yield other extractives of a crystalline nature, probably salts of amidoacids. Of the presence of the latter I have not been able to satisfy myself. The question, however, is a complicated one and must be regarded as still undecided. I shall have occasion to refer again to these substances when dealing with the clinical aspects of the subject.
II CLINICAL

I shall now proceed in the light of these chemical results to attempt to reply to the question - What is the active substance contained in the thyroid? And, first, what do we mean by the 'activity' of the thyroid? I take it that the results of the therapeutic administration of thyroid preparations completely disprove the old theory which regarded that gland as chiefly concerned with the removal of some deleterious material from the blood, and that they have definitely proved that the thyroid contains within itself some substance or substances, the presence of which in the body is necessary for the normal march of metabolism. If this substance be present in normal amount we have a state of health. If it be deficient, the symptoms of myxoedema or cretinism develop. If it be in excess, we get what has recently come to be known as 'thyroidism'. The removal of the symptoms of myxoedema or cretinism by the administration of any thyroid preparation or the production by it of the signs of thyroidism, is the only method we have of telling whether that preparation is or is not active. The symptoms of thyroidism vary in different individuals being apparently largely
dependent upon idiosyncrasy. They would appear to be much more easily produced in the subjects of myxoedema and cretinism than in normal individuals. Speaking generally, they consist of (1) an increased rate of metabolism manifesting itself by slight rise of temperature and progressive loss of weight, and a greatly increased excretion of nitrogen which may lead to polyuria, (2) an increased rapidity of heart beat and the not unfrequent appearance of cardiac irregularity (3) general disturbances - restlessness, morning headache, myalgic pains, excitability or depression of spirits, tremulousness and occasional albuminuria or glycosuria.

These symptoms are not by any means always present at one time. In one case one set of symptoms may predominate, in another case, another, and there would appear to be some healthy individuals who are capable of taking very large doses of thyroid preparation without suffering from any of them at all. It has also been frequently observed that the active substance of the thyroid has a cumulative action - the symptoms persisting for some days after administration has ceased. Numerous speculations have been advanced as to the nature of the substance which produces these
symptoms of thyroid activity. Some have supposed it to be a ferment. Gourley suggested that it was a nucleo-albumin. Baber (3) arguing on histological grounds, was of the opinion that it was the colloid matter. Recently Baumann has asserted that it is his thyroiodin, Fraenkel that it is his thyreo-antitoxin and Drechsel that it is probably one or both of the bodies isolated by himself.

The only satisfactory way to attack the problem would appear to be by isolating the chief chemical ingredients of the gland and observing the effects of the clinical administration of each separately. A substance which is capable in a normal individual of producing distinctly some of the symptoms of thyroidism may provisionally be regarded as active. If the same substance exerts a curative effect in myxoedema or cretinism its activity may be regarded as certain. It is upon these lines that the observations which follow have been conducted.

I first made some preliminary observations on three patients who were under treatment for psoriasis and the effect in whom of the administration of thyroid tabloids had already been ascertained. By extraction of the tabloids with water and with rectified
spirit, I removed from them everything except proteids and a little fat. I found that the dry powder so obtained was still active and concluded that the active ingredient of the thyroid was a proteid and not an 'extractive'. I then proceeded to isolate the two chief proteids of the thyroid - the colloid and the nucleo-albumin - in the manner already described, the extract after the removal of these was evaporated to a small bulk and kept. I thus obtained three preparations (1) the dried colloid, (2) the dried nucleo-albumin, (3) a proteid-free watery extract. The first I have always administered in the form of powder without any addition. The nucleo-albumin, owing to its very small bulk, was mixed with milk sugar and then given, also in the form of powder. The concentrated extract was made up with water to a definite volume and shaken up with a few drops of chloroform to improve its taste and to aid its preservation; it was then administered in doses of $\frac{3}{4}$ or upwards, the equivalent of the dose in grammes of fresh gland being easily calculated.

In order to give these preparations a fair trial it would be well, of course, to exhibit them in cases of myxoedema or of cretinism. These, however,
are not always to be obtained. I have only been able to try them (1) in one case of myxoedema, which, however, was already partially cured, (2) in a case believed to be one of mild myxoedema, but the real nature of which I think was somewhat doubtful. (3) in a case of exophthalmic goitre. Full details of these cases with charts etc. will be found in the accompanying protocols but I propose here to focus the chief points of each case and the results of the treatment upon it, and to state the inferences which one was able to make from these results as to the nature of the active ingredient of the thyroid.

Case I.

Mrs P. (54). This was a case of myxoedema of some years duration. Unfortunately the patient did not come under my observation till she had been already six weeks under treatment by thyroid tabloids. Under this treatment she lost one stone, eight and a half pounds in weight, the pulse rose from about 50 to 64 per minute and the temperature from being sub-normal became normal or slightly irregular (see chart). I cannot speak from my own observations as to what symptoms of thyroidism (pains, headache etc.) she may
have exhibited during this treatment. When she came under my own observation many of the symptoms of myxoedema had already passed off but she was still far from being in a normal state (see photo). Treatment by the tabloids was stopped and the patient was left for a few days untreated. She immediately began to gain again in weight, the temperature fell somewhat and the pulse averaged in the morning 64 and in the evening 60.6

She was then given 0.1 gramme of dried colloid thrice daily. As a result she immediately began to lose weight again, the temperature rose slightly and the pulse for the first week of treatment averaged in the morning 69.1 and in the evening 67 (an increase of 5-6 beats). She had also some symptoms of thyroidism - aching pains, frontal headache, palpitation etc. (see note for January 31st). The treatment by colloid was continued as described in detail in the case notes for four weeks. At the end of that time she had improved greatly in appearance and in intelligence (see photo). She had lost in weight since the colloid was started four weeks previously 15 1/2 lbs. - an average of nearly four pounds per week. Comparing this with the results of the treatment by thyroid
tabloids we find that she lost upon these 20½ lbs. in six weeks, or an average of 3.4 lbs. per week. The loss of weight, therefore, was greater in the case of the colloid than in that of the thyroid tabloids (for relative sizes of dose of these see chart). The effect upon the pulse rate was also striking. Under the treatment by the tabloids, it rose from 50 on admission to about 64. The average morning and evening pulse rate for each week of the colloid treatment is represented in the following table.

<table>
<thead>
<tr>
<th>3 days without treatment</th>
<th>1st week of colloid</th>
<th>2nd week</th>
<th>3rd</th>
<th>4th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morning</td>
<td>64.3</td>
<td>69.1</td>
<td>76</td>
<td>80.4</td>
</tr>
<tr>
<td>Evening</td>
<td>60.6</td>
<td>67</td>
<td>69.1</td>
<td>70.8</td>
</tr>
</tbody>
</table>

It will be noted that a steady increase in the rapidity of the pulse took place. The effect upon the temperature is shown in the chart.

We learn from this case, then, that the colloid matter even in moderate doses is capable of producing all the symptoms which are produced by the administration of the entire thyroid - progressive loss of weight, rise in temperature and pulse rate - headache myalgic pains, palpitation etc. and, further, that it has a curative influence in myxöedema. The conclusion
is therefore justified that the colloid matter is at least an active constituent of the thyroid.

Case II.

This was the case of a woman aged 29 who presented several of the symptoms of myxoedema without, however, being by any means a typical example of that disease. The clinical history and symptoms will be found in detail in the protocol. She was treated on the assumption that she was suffering from myxoedema. She was first kept under observation for seven days without treatment, she was then put upon thyroid tabloids and the effect of these ascertained. The fluid extract (proteid-free) of thyroids was then exhibited and its effects noted and lastly the colloid matter was given. During the seven days that the patient was under observation before treatment began, she lost 2\(\frac{1}{4}\) lbs. in weight, the temperature was slightly irregular at times, the pulse rate will be found in the table. Under the thyroid tabloids the temperature became more irregular (see chart) and the pulse rate increased (see table) and she lost two pounds in weight. There were no other symptoms of 'thyroidism'. She was then put upon the fluid extract of thyroid which contains no proteids but all the extractives of the thyroid.
<table>
<thead>
<tr>
<th>Pulse</th>
<th>7 days before treatment</th>
<th>2 tabloids thrice daily</th>
<th>3 tabloids thrice daily</th>
<th>Extractives thyroid</th>
<th>Colloid 0.3</th>
<th>Colloid 0.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morning</td>
<td>74</td>
<td>72 8</td>
<td>86.6</td>
<td>79</td>
<td>83</td>
<td>89</td>
</tr>
<tr>
<td>Evening</td>
<td>78</td>
<td>77</td>
<td>92</td>
<td>79</td>
<td>90.6</td>
<td>96</td>
</tr>
<tr>
<td>Weight</td>
<td>lost 2 lbs.</td>
<td>lost 1 lb.</td>
<td>lost 1 lb.</td>
<td>gained 1(\frac{1}{2}) lbs in 10 days</td>
<td>lost 4 lbs. in 10 days.</td>
<td></td>
</tr>
</tbody>
</table>
It would therefore contain the bodies of Fraenkel and Drechsel. This preparation showed itself entirely inactive although the patient got in 10 days as much of it as would represent 60 grammes of fresh thyroid (rather more than one sheep's thyroid per diem). She gained weight under it (see chart) and pulse rate fell (see table) the temperature, also, became more steady (see 4-hourly charts). She was then put upon the 'colloid' in doses of 0.3 grammes night and morning. As a result the temperature at once became somewhat irregular again and pulse rate rose, the patient also began to lose weight and suffered from myalgic pains, headache, palpitation and tremulousness (thyroidism).

The average pulse rate and weight under the different forms of treatment is represented in the table on the opposite page.

The case shows that the colloid matter produced all the symptoms - and in an exaggerated form - which were produced by the tabloids. The colloid is therefore active. On the other hand, no symptoms at all were produced by the solution of the extractives of the thyroid. I therefore conclude that these are inactive.
Case III

This was a case of exophthalmic goitre of five years' duration occurring in a young woman. The ordinary symptoms were present but in a by no means exaggerated form (see notes of case). In addition there was slight cardiac irregularity but without any sign of valvular disease. There was, however, a history of rheumatic fever.

The thyroid treatment having been resolved upon, the patient was first kept for ten days under observation before anything was tried. During this time the temperature was slightly irregular (see chart) and she lost about 2 lbs in weight. The average pulse rate was 90 - slight irregularity and inequality but no intermittency was present. Dried thyroid nucleoalbumin was then begun in doses of 0.02 grammes thrice daily (equal at least to 6 grammes fresh thyroid per dose). This was continued for 5 days. The result was entirely negative - the body weight remained practically unaffected, there was no rise of temperature, no complaint of any sort of discomfort or disturbance, the pulse average during the five days remained at 90 and its characters were unaltered.

The treatment was then changed to the
administration of the liquid extract of thyroid (proteid-free). Of this she got \( \frac{1}{2} \) an ounce (= 3½ grammes fresh thyroid) thrice daily for a week. Again the result was entirely negative. Body weight, temperature and pulse rate remained as before and no symptoms of thyroidism appeared. She was then put upon 0.1 gramme of dried colloid morning and evening. After four doses had been taken she complained of violent palpitation and oppression at the heart. The pulse became extremely feeble and intermittent and the symptoms were so alarming that the administration of the colloid was at once stopped. The palpitation and intermittency of pulse lasted for four days after the discontinuance of the treatment, after which she gradually returned to her usual condition. Another interesting result of the colloid was the appearance of sugar in the urine. This was first observed on the day after the bad effects of the substance were first complained of and persisted for four days, gradually disappearing. The temperature was not appreciably affected in this case and it was not considered safe to remove the patient from bed in order to weigh her. The chief incidence of the effects of the colloid in this case seems to have been upon the heart, with the
exception of slight pains in the legs no other symptom of thyroidism was complained of.

I am not able in this case, as I was in the others, to compare the action of the colloid with that of the thyroid as a whole, as the effects of the remedy on the heart were so severe as to render it unjustifiable to attempt the administration of thyroid tabloids even after the effects of the colloid had passed off. I think, however, that there can be no question of the activity of the preparation. Its effects are all the more striking after the entirely negative results of the administration first of the thyroid nucleo-albumin and secondly of the solution of thyroid extractives, and afford additional evidence that the colloid matter is the only part of the thyroid possessed of clinical activity.

It would be difficult to say whether the severe action upon the heart in this case is or is not to be attributed to the fact that the case was one of exophthalmic goitre. It must, of course, be borne in mind that the action of the heart was always irregular. The production of glycosuria by the colloid matter is also of interest as that phenomena has been observed to occur occasionally in the course of ordinary thyroid treatment.
From the results afforded in these cases, I think that one is entitled to conclude (1) that the colloid matter when isolated in a state of purity is capable when administered by the mouth (1) of producing the usual symptoms of thyroidism (progressive loss of weight, headache, myalgic pains, tremors etc) and (2) of exerting a curative effect in myxoedema. That is to say, it possesses the active properties characteristic of the thyroid. It is possible, however, that there may be other active substances present in the gland as well as the colloid matter. Have we any evidence of the existence of such? Fraenkel (op cit.) states that he administered to a patient the proteids from seven thyroids every day for ten days and found no loss of weight. He then tried the neutralised filtrate (proteid-free) on himself and found a loss of weight amounting to 300 grammes per day for six days. This was accompanied by an increase in the pulse rate. He got the same result in a case of obesity but he gives no details of his observations. He concludes that the proteid free filtrate contains the active substance. With this result my own observations are entirely at variance. As stated above, I administered the proteid free extract in case II in very large
doses, the patient receiving as much as would represent at least two sheep's thyroids a day for a week without any loss, indeed with a gain, of weight, nor was there any acceleration of pulse. Yet under the tabloids and under the colloid even in moderate doses, the loss of weight was marked and progressive and the pulse acceleration quite distinct. (These observations were confirmed in case III). I cannot, therefore, admit that the thyroid contains an extractive or alkaloid - call it what you will - which is possessed of clinical activity until stronger evidence than that furnished by Fraenkel has been adduced (of experimental evidence I shall speak later). As the watery extract which I used would also contain the body isolated by Drechsel I am of the opinion that it also is not possessed of any clinical activity. Of the other proteid of the thyroid - the nucleo-albumin - the same must be said; I have not, as stated before, been able to collect enough to give it an extended trial but in the one case in which it was exhibited it showed no unequivocal signs of activity. Yet a substance present in so very small amount would require to be extremely potent did the thyroid owe its activity to its presence. I conclude, then, that the colloid
matter is the only clinically active ingredient of the thyroid. To further substantiate this proposition one must show that all thyroid preparations hitherto found to be active, contain the colloid matter. This, I believe, I am able to do. Of the dried preparations of the whole gland, e.g. thyroid tablets, one need not speak. The liquid extracts of the thyroid made with water, or with water and glycerine, also contain the colloid in solution provided they have not been boiled, and I am not aware of the existence of any boiled extract which has been found to be active. There is one preparation which demands special mention. White, the pharmacist to St. Thomas' Hospital, acting on the belief that the thyroid might owe its activity to the presence of a special ferment produced in a glycerine extract of the thyroid a precipitate which would contain the ferment if it existed. The precipitate he produced was one of phosphate of calcium, that being a gelatinous precipitate specially suited to carry down the ferment. The precipitate when dried was found to be active and has been pretty largely used in the treatment of myxoedema and cretinism. I have prepared this precipitate according to the directions given by White. To an extract

Colloid is present in all the preparations hitherto found to be active.
of sheep's thyroids phosphoric acid was added in the proportion of 55 minims of the strong acid to one pint of extract. Lime water was then added to neutralisation. A dense precipitate was produced and was filtered off. The filtrate was found to contain no colloid matter the latter having been evidently thrown down along with the phosphate of lime. White states that his powder contained 50% of organic matter. There can be no doubt that this consists largely of the colloid. On the other hand, I can see no reason why crystalline and soluble substances such as those described by Fraenkel and Drechsel should be thrown down in that manner and I would conclude that the powder prepared by White owes its activity to the fact that it contains the colloid matter.

As far as I am aware, the only other thyroid preparation in addition to these already mentioned, which has shown itself possessed of clinical activity, was a precipitate produced by the addition of absolute alcohol to a glycerine extract of thyroids. Vermehren, in the Dent. Med. Wochenschrift for March 16th 1893, describes a case of cretinism cured by the giving of this precipitate. Obviously such a precipitate will contain the proteids of the thyroid including
the colloid matter. As Fraenkel's body was soluble in alcohol it would not be contained in the precipitate. This is another argument against the view that his "Thyreo-antitoxin" is the active substance in the thyroid.

If the colloid, then, is to be regarded as the only active ingredient of the thyroid the question arises, is the colloid active as a whole or does it only contain some active substance within it? I have shown that the colloid matter is to be regarded as a compound proteid and that it can be split up into (1) a proteid part and (2) a part free from proteid matter altogether. The former is probably a globulin, the latter is a peculiar substance characterised especially by the presence in it of iodine. Now Baumann (op. cit) as has been already stated, has isolated from the whole thyroid an organic compound of iodine which has been found by Roos who tested it to be possessed of clinical activity. At the time of writing, these clinical results have not been published, but if the observations of Roos are correct it would indicate that the colloid matter owes its activity to its proteid-free or iodine containing part, for the colloid, as I have shown, is the only iodine containing part of
the thyroid. I have not yet myself been able to investigate the clinical effects of giving the proteid-free part of the colloid by itself, all of the substance which I have been able to prepare having been required for purposes of chemical investigation. There is, however, great probability that this view is correct. The presence in the colloid of this iodine-holding body being its chief peculiarity, the latter may be not unnaturally regarded as the cause of the peculiar effects produced by the colloid as a whole. It may be of interest in this connection to state that if one incinerates, with proper precautions, even a single one of Burroughs' and Wellcome's tabloids, the presence of iodine can be easily demonstrated in the ash.

What follows from regarding the colloid matter as the active ingredient of the thyroid? Obviously the first result of such a conception is the harmonising of the teachings of histology with those of chemistry. Baber (3) Langendorff (5) and Hürthle (6) had already inferred from the study of the histology of the thyroid gland that the colloid matter was to be regarded as its secretion - produced by the cells lining the alveoli and carried away into the circulation by the lymphatics. This inference, chemical
investigation fully endorses and thereby brings the Thyroid into line with other secreting glands. And what follows from regarding the iodine containing substance as the part of the colloid matter to which the latter owes its activity? The consequences of this conception are many and may ultimately be found to throw light upon many chemical processes in the body. In considering its bearings upon these, a wide and tempting sea of speculation opens out upon which, however, I do not propose to embark. I shall confine myself to some remarks upon the light which these new conceptions of the physiological product of the thyroid throw upon some diseases associated with that gland. In the first place one cannot help remembering the use of iodine in the treatment of goitre. But here one must enter a caveat. There is really no reason why the administration of a mineral substance contained in the secretion of the thyroid should, on that account, be expected to have any beneficial effect on enlargements of that organ. And more than this, the colloid matter cannot be believed to owe its activity to the presence of iodine qua iodine. The amount of the latter in the colloid is altogether too small to make that view admissible. Rather must it
be due to the special form of combination in which the iodine occurs in the colloid that the latter is active.

On the other hand, if it be the case, as one is compelled to believe it is, that the cells of the thyroid gland are endowed with an affinity for iodine so great that they can pick it out from the blood even in the minute quantity in which it must normally be present in that fluid, it seems probable that the amount of iodine present in the food cannot be without influence on the amount of colloid produced. It would perhaps be worth while to investigate whether there is any traceable connection between the presence of an excess of iodine in, say, the wells and springs of a district and the existence in that district of goitre in an endemic form.

The precise rôle which the thyroid plays in the production of such diseases as myxoedema, cretinism and exophthalmic goitre can only be properly appreciated when we have a more complete knowledge of what one might call the pharmacological action of the colloid matter than we at present possess. The nature of such action in the human subject can only be inferred from the effects produced by the clinical administration of the colloid and of these we have.
already spoken. They may be summed up by saying that the colloid matter when it enters the circulation acts as a stimulant to proteid metabolism - probably by reason of the iodine holding substance which it contains. All the symptoms of "thyroidism" can be traced to this increased proteid metabolism. It explains the increased output of nitrogen, the loss of weight, the slight pyrexia and the hurried on of the heart's action. The associated symptoms, - headaches, muscular pains etc. are not improbably toxic in their nature - due to the circulation in the blood of the products of the increased tissue waste. In myxoedema and cretinism the thyroid has ceased to manufacture the colloid matter. This, morbid anatomy has already told us and chemistry correlates the fact for us with the production of the symptoms of the disease. For the essential feature in myxoedema is a slowing of the general metabolism and to this it is probable that all its symptoms can be attributed. The pathological fact to which the disease owes its name, the occurrence of a myxomatous thickening of the subcutaneous tissue is apparently to be attributed to an arrested development of the subcutaneous tissues and their failure to pass on into a fibrous condition. This
comparatively embryonic tissue, like all young tissues, is relatively much richer in mucin than fully formed tissues are - hence the fact that the subcutaneous tissue in myxoedema is richer in mucin than that of health (see Halliburton's Analyses, Text-book of Physiological Chemistry, p. 506). It was no doubt owing to the close physical resemblances of the colloid matter to mucin that the thyroid was so long supposed to act as a destroyer of the latter substance.

When the colloid matter is administered in a case of myxoedema, the spur to metabolism which was wanting is now supplied and the further stages in the life history of the arrested tissues are rapidly completed.

The sudden disintegration of these embryonic tissues must flood the circulation with the products of their waste and these, as we have indicated, are probably to some extent of a toxic character. It may perhaps be due to the large amount of such products which enter the circulation that the results of thyroid treatment in myxoedema are so much more severe than in a state of health. The fully formed tissues which predominate in health are probably more resistant to the action of the colloid than young tissues are. Hence the administration of thyroid in such a case will lead to
less tissue waste and fewer waste products will enter the circulation; thyroidism, therefore, will be less likely to occur. If this be not the true explanation of the difference to be observed in the results of thyroid feeding in myxoedema and in health, one must assume that there is at work in the body, some agency antagonistic to the action of the colloid and that in myxoedema this antagonism has, for some reason or another, ceased to be exerted.

The conception of the colloid matter as the active ingredient of the thyroid cannot be said to throw much light on the pathology of exophthalmic goitre. Into the controversies which have raged, and are now raging, around the question of the relationship of the thyroid gland to that disease, I do not propose to enter. I shall confine myself to the purely chemical aspects of the question. After the results of Greenfield's investigations into the microscopic changes in the thyroid in exophthalmic goitre, one is almost forced with him to the conclusion that the changes met with in the thyroid in that condition are analogous to those found in glands undergoing evolution for increase of function. But if the thyroid be a secreting gland 'increase of function'...
must mean increase of secretion, and if the colloid matter be the substance secreted, there ought to be an evident increase in the amount of the colloid matter in exophthalmic goitre, but this apparently does not occur. In addition to this, there is no evidence that the experimental administration of thyroid leads to the production of anything which can be identified with Graves' disease. Ballet and Enriquez (8) are the only writers, so far as I am aware, who have reported the production of any exophthalmos at all by artificial hyperthyroidisation. This, they state, occurred in one rabbit to some extent. The same writers claim to have produced also some enlargement of the thyroid by the same means. This, they succeeded in doing in three cases in dogs. The microscopic condition they state to have been the same as that which they believe to characterise the thyroid in Graves' disease - an obliteration of the intralobular lymphatics by inflammatory reaction.

Of the results of the administration of thyroid to patients suffering from Graves' disease, I have not been able to find many reports and I have only had one case of it under treatment with the colloid matter. Edmonds (9) in a recent paper states that cases do not
get worse under it. If this be found to be the case it is against the view that Graves' disease is due to an over-production of the normal secretion of the thyroid (i.e. of the colloid matter). The possibility of an abnormal secretion must always be borne in mind but I have had no opportunity of investigating the thyroid from a case of Graves' disease chemically.

I may now pass to a brief consideration of the bearing which the views upon the nature of the active substance of the thyroid, which I have endeavoured to put forth, have upon some of the results obtained by experiment upon animals. I have myself made no experimental observations upon the action of the colloid matter. It seems to be pretty universally agreed that in all the lower mammals, at any rate, dogs, cats, rabbits and the like, the symptoms which follow the removal of the thyroid are much more acute and severe than those ever observed in man; and further, it has been found difficult or impossible to save the lives of such animals even by the administration of the thyroid as a whole (see on this point the papers of Edmunds (9) and of Ballet and Enriquez (8) already referred to). The observations which I have made on the colloid matter and its clinical effects, throw no
light on these results. It is, of course, always a possibility that the ingredients out of which the colloid is produced are themselves harmful and that the thyroid not only manufactures a useful substance but succeeds in doing so out of dangerous elements. This would explain the increased toxicity of the blood serum of the dog after removal of the thyroid which has been insisted upon by Gley (10) and by Masoin (11). It must be admitted, however, that the facts of myxoedema and the results of its treatment do not favour this view.

Leaving these general considerations, one may pass to some specific observations of various writers and their bearing upon the thesis I am endeavouring to maintain. And first, one may refer to the work of Notkine (12). Notkine asserts that he has isolated from the thyroid 'a proteid which constitutes the chief bulk of the colloid matter' and which 'he believes to be the cause of cachexia strumipriva'. Its administration in large doses to animals from whom the thyroid had been removed, was found by him to cause death in a few hours with the appearance of dyspnoea and convulsions. In healthy animals it produced a more gradual train of symptoms (including general
weakness, emaciation, dyspnoea) ending either in death, or, in the case of smaller doses, in a chronic intoxication. He asserts that the action of this proteid on animals is first exciting and then paralysing, the heart being weakened and slowed. He believes that it is the function of the thyroid to destroy this substance or to neutralise it by means of its true secretion which, he apparently considers to be of the nature of a ferment.

I have already dealt in detail with the nature of the colloid and with its chemistry and I am entirely at a loss to understand how Notkine gets from it any body such as he describes. I am at a still greater loss to understand how the results produced by the experimental administration of such a body to animals, should be so entirely at variance with the symptoms produced by the colloid in man. Further criticism, however, is useless until Notkine publishes further chemical details as to his proteid body and the mode of procuring it.

One must refer, in the second place, to the experimental results of Fraenkel. As already stated, he has isolated a substance, apparently alkaloidal in nature, to which he has given the title 'Thyreocentitoxin.'
He finds that this substance when injected intravenously in animals produces a fall of blood pressure and an increased rapidity of pulse. Schäfer(14) has found similar effects on injecting a boiled extract of thyroid. These are, of course, the chief circulatory effects observed as to the result of the clinical administration of thyroid preparation. It would be rash, however, to conclude that it is the presence of Fraenkel's substance in the thyroid which enables the latter to produce these effects. The effect of ordinary extractives, such as creatin on the blood pressure and pulse would first require to be ascertained and one would require to show that the thyroid is the only gland, - a decoction of which, when injected intravenously, produces these results. Schäfer, indeed, has admitted that this is really not the case but that a decoction of salivary glands produces the same effects. Nor can I attach much value to the effect which Fraenkel found his body to have on kittens from which the thyroid had been removed. He states that his antitoxin was able to prevent the convulsions which supervene in such animals, but was not able to ward off death. These results cannot be regarded as conclusive - the prevention of
convulsions might be due to many things, for example to the lowering of the blood pressure which he has shown his substance to produce. I can only repeat what I have already stated - that until Fraenkel's antitoxin has been clearly shown to be capable of producing the symptoms of thyroidism or of exerting a curative effect in myxœdema, it cannot be regarded as the clinically active ingredient of the Thyroid.

The effects of the experimental administration of the colloid matter upon thyroidectomised and upon normal animals I propose to study when time and opportunity offer. I shall then be in a position to compare the results with those of other observers obtained by different methods. The object of the present investigation, however, was different - to ascertain by clinical observation what is the ingredient of the thyroid which produces the effects commonly recognised as resulting from the administration of thyroid preparations. In that object I believe that I have succeeded. I think that I have been able to show:-

(1) That the colloid matter and it alone, is the active ingredient of the thyroid, and
(2) That the colloid matter probably owes its
activity to the presence in it of a peculiar body which is specially characterised by the fact that it contains iodine in organic combination.
PROTOCOLS OF CASES.

referred to in the text.
(Myxoedema)

Showing results of treatment by the colloid matter of the thyroid. (This case did not come under my own observation until six weeks after admission to Hospital).

Mrs. P. Aet. 54 was admitted to Hospital on December 10th, 1895 complaining of swelling of the face, hands and feet of four years duration. There was nothing noteworthy in the history. The symptoms had come on gradually about the time of the menopause.

From the notes made at the time of admission she seems to have presented in a fairly typical form, most of the symptoms of myxoedema. The general hebetude and slowness of speech seem to have been fairly well marked. The skin was rather harsh, the hair fairly abundant on the scalp but dry, that of the eyebrows very scanty. Subcutaneous thickening was evident in the face and hands, the latter being rather spade like.

No note was made of the condition of the thyroid. Otherwise the organs were normal. The weight on admission was 13 stones 5½ lbs. The temperature sub-normal. The pulse rate 52.
Progress.

During the first three days in Hospital she gained 3 lbs. in weight. She was then put upon thyroid tabloids (one thrice daily). This dose was afterwards reduced (see charts). This treatment was continued for six weeks. At the end of this time marked improvement had resulted. The patient had lost 1 stone 8 lbs. in weight and was evidently brighter and more intelligent than before. The subcutaneous thickening had largely disappeared. The pulse rate had risen to 64 and the temperature was now normal. It was at this time that the patient first came under my own observation. It was noted that there were still present some slowness of speech and movements but there was now very little subcutaneous thickening and the hands had lost their spade-like character. The skin was smooth but rather dry, the hair of the scalp was fairly abundant; that of the eyebrows still very scanty. The pulse rate was 64.

The accompanying photograph exhibits the appearance of the patient at this stage.

Further Progress.

On January 22nd treatment by thyroid tabloids
was stopped. As a result the patient's weight began immediately to rise and during the next three days she gained 1 lb. per day. The temperature remained sub-normal. The average morning pulse rate for the three days was 64; evening 60.

On January 26th treatment by means of the colloid matter of the thyroid was begun, 0.1 grammes (about 2 tabloids) being given thrice daily, and on January 28th the dose was doubled. The notes now proceed as follows.-

Jan. 31st  Patient feels very ill, complains of severe general pains of an aching character in the back and limbs. Frontal headache was present in the morning. She has had some palpitation. Pulse 74, regular. Volunteered the statement that she feels "just as she did when on large doses of the tabloids." Colloid reduced to 0.1 daily.

Feb. 2nd  Feels much better since reduction of dose, pains and headache gone, no palpitation.

" 3rd  Colloid increased again to 0.1 grammme thrice daily.

" 5th  Some aching and stiffness have returned in
Case I (Mrs P.) before treatment with "Colloid"

The Same - after 4 weeks' treatment with Colloid matter of Thyroid.
the limbs, otherwise as before.

Feb. 6th Feels very ill again - pains increased - some faintness - pulse feeble but regular (82) Colloid stopped.

11th Above symptoms have gradually passed off but has lost 4 lbs. of weight since last note. Colloid begun again.

20th Since last note has been taking 0.3 grammes colloid in a single dose at mid-day. Has lost weight steadily and pulse rate has increased but makes no complaint of pains. Colloid increased to 0.4 grammes.

24th Aching pains have returned again and has lost 2½ lbs. of weight in last four days. Colloid stopped entirely.

29th Left Hospital. accompanying photo shows state on leaving.

The chart shows the daily temperature variations and the alterations in weight throughout the period of treatment; also the morning and evening pulse rate since the patient came under my own observations. For comments upon the case see text.
CASE II.

(?Myxoedema.)

Mrs F. (29)

Admitted to Hospital January 15th 1896 complaining of weakness and headache of twelve months duration.

There was nothing noteworthy in family or personal history. During last two years the patient had become much stouter and her hair has come out in considerable quantity. Her memory also has failed somewhat and she notices that her speech is slower than it used to be.

On physical examination the patient was found to be a well developed woman with a slight excess of subcutaneous fat but no true myxoedematous condition of skin, which was moist. Her complexion was pale without any ruddy spots on cheeks. Her manner was rather dull and listless, the temperature normal, pulse 88. All the systems were normal. The thyroid could not be felt but there was a large amount of subcutaneous fat in the neck.

Progress.

Kept for a week under observation. Temperature
<table>
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<tr>
<th>Date of Admission</th>
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<td>Date of Discharge</td>
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**Case II**

**Diseases:**

- Hyperpyrexia
- Pyrexia
- Normal Pulse

**Condition of Tongue, Abdomen, Bowels, Urine, Skin, Nervous Symptoms:**

- Tongue: Normal
- Abdomen: Normal
- Bowels: Normal
- Urine: Normal
- Skin: Normal
- Nervous Symptoms: Normal

**Temperature Scale:**

<table>
<thead>
<tr>
<th>Date</th>
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<tr>
<td>12/27/21</td>
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<td>99°F</td>
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<tr>
<td>12/31/21</td>
<td>98°F</td>
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</table>

**Other Observations:**

- Febrile diaphoresis
- Lethargy
- Nausea
- Vomiting

**Prescriptions:**

- Patient is on a liquid diet
- Antibiotics

**Suggested by Dr. R. G. Patteson:**

**Printed and Published:**

- Van Houten & Co., 52 East 13th Street, New York, N.Y.
continued slightly irregular (see chart) and she lost 2 lbs in weight. Nothing else noteworthy.

Jan 25th  One thyroid tabloid, (5 grains) thrice daily.

" 28th  Increased to two tabloids.

Feb  2nd  Complains of pains in limbs and some frontal headache. No other symptoms of thyroidism.

" 4th   Three tabloids thrice daily.

" 10th  Frontal headache and giddiness, pain in limbs worse. Losing weight. Pulse rate increased (see chart). Tabloids stopped.

" 17th  Above symptoms have all passed off - Pulse rate has fallen again. Began extractives of thyroid (3/ of liquid extract thrice daily = 1.5 grammes fresh thyroid).

" 23rd  Been on 'extractives' for a week. Feels very well. No symptom of thyroidism. Is gaining weight and pulse rate has fallen.

Mar  2nd  Still no symptoms. Extractives stopped.

"  3rd  Began 0.3 grammes colloid night and morning.

"  6th  Feels 'sore' all over and has frontal headache, slight palpitation this morning, pulse rate raised (see chart) and has begun
Case II. Hourly charts for the purpose of comparing the effect when the temperature (1) of thyroidal (2) or its "extractives" (3) of its "colloid" is used for the administration. No or slight increase here which is given
to lose weight again.

Mar 8th  Colloid increased to 0.4 grammes morning and evening.

" 10th  Still has muscular pains and headache. Movements are slightly tremulous.

" 13th  Pains and headache more severe, movements now extremely tremulous, pulse feeble and slightly irregular. Been very low-spirited during last three days. Urine still normal. Colloid stopped.

" 17th  Left Hospital. Has gained a little in weight since 'colloid' was stopped. All symptoms of thyroidism have disappeared but pulse still rather rapid. Is much brighter and more active than on admission.

(For detailed register of temperature, pulse etc. see accompanying charts).
CASE III.

(Exophthalmic Goitre.)

Shewing effects of nucleo-albumin, extractives and colloid matter of thyroid.

Agnes Thomson (27)

Admitted March 2nd. 1896.
Complaint - prominence of eyes and palpitation.
Duration - 5 years.

Family History - nothing noteworthy.

Previous Health - Rheumatic fever 10 years ago and again 3 years ago - never well since last attack. No other illnesses.

Present Illness. Swelling in neck was noted about 5 years ago. Eyes became prominent about the same time. These symptoms have continued with slight fluctuation since then; has occasional attacks of palpitation.

Present State. Spare, complexion shallow, slight prominence of eyes - sweats easily - weight etc. (see chart).

Circulatory System Heart.

Occasional palpitation rhythm slightly unequal and irregular. No enlargement of heart, sounds pure.
Pulse 96, unequal, irregular, small, soft.

Thyroid Considerably enlarged, asymmetrical right lobe larger. Marked pulsation and slight thrill. Circumference of neck at 6th cervical vertebra = 13½ inches.

Eyes Slight exophthalmos; Von Graefe's symptom present.

Urine normal

Progress

Mar. 10th Up till now no treatment. Has lost 2 lbs in weight since admission. Pulse rate has averaged about 90, it remains somewhat irregular and unequal. Temperature also somewhat irregular. Urine normal; no complaint of any special symptom. nucleo-albumin begun in doses of 0.02 grammes thrice daily.

" 13th No change.

" 15th Nucleo-albumin stopped - has produced no appreciable result. Half an ounce of liquid extract of thyroid (proteid-free) thrice daily. (each dose = 3½ grammes fresh gland).

" 18th Quite well; no result from extract.
### Case II

**Disease:**

- Bowel

**Notes or Case:**

- Age
- Date
- Case No.

**Date of Admission:**

**Result:**

**Printed and published by Worlderspoon, 797 7th Street, Lincoln, Nebr.**

**Goulds Clinical Chart: State 4. Printed and published by Worlderspoon, 7th Street, Lincoln, Nebr.**

<table>
<thead>
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<th>Time</th>
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**No treatment**
Mar. 21st  Still no symptoms at all; pulse etc. as before. Extract stopped.

" 23rd  Colloid begun in doses of 0.1 grammes morning and evening.

" 25th  Did not see patient again until to-day. Since evening of 23rd has had frequent attacks of palpitation; last night these were very severe. Pulse now 84; extremely small; irregular and intermittent. Seems rather anxious and excited. Complains of pains in the legs but has no headache. Exophthalmos seems less pronounced. Circumference of neck at level of 6th cervical vertebra = 13½ inches. Urine not examined. Colloid stopped.

" 26th  Palpitation still present and pulse has same characters as yesterday. Urine increased in amount and contains a considerable amount of glucose (no glycuronic acid).

" 27th  Pulse still very small, irregular and intermittent. Palpitation less severe. Urine has sp. gr. of 1020 but still contains sugar.

" 29th  Palpitation much less; intermittency of
pulse almost gone; only a trace of sugar in urine.

* 30th Pulse has returned to its original character; palpitation and glycosuria have ceased. Thyroid treatment not to be recommenced.
LIST OF WORKS

referred to in text.

-----oo0oo-----
8. Dallet et Enríquez: La Semaine méd. Aug. 7th 1895 (Full discussion in same number)
11. Mason:
12. Hotline: La Semaine méd. April 8th 1895

For reports of work on physiological action of thyroid extract, the following references may be made to:

