OBSERVATIONS

ON THE

FUNCTIONS OF THE LIVER,

MORE ESPECIALLY WITH REFERENCE TO

THE FORMATION OF THE MATERIAL

KNOWN AS

AMYLOID SUBSTANCE, OR ANIMAL DEXTRINE,

AND THE

ULTIMATE DESTINATION OF THIS SUBSTANCE
IN THE ANIMAL ECONOMY.

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PREFACE.

THE majority of those engaged in practice, still, I believe, look upon the liver as if the principal duty of this gland was nothing else than the secretion of bile. It is certain, however, that it does other work, little, if at all, inferior in importance to the formation of biliary matters, and quite as necessary to the maintenance of health. Its power of making, and storing up for a time within its cells, a material resembling starch, constitutes, without doubt, one of its most important functions. This no person will for a moment doubt, who takes the trouble of ascertaining, by experiment, the immense increase or diminution in bulk which the liver may be made to undergo in the space of a few days by such changes of diet as increase or diminish the amount of this starch-like material in its tissue.

The subject is one which I conceive to be of great interest, in a practical point of view; and on this ground I would solicit the attention of practitioners to the *facts* recorded in the following pages, especially those relating to "The Formation by the Liver of Amyloid Substance" (p. 11, and seq.). While I venture to hope that physiologists will also carefully consider the facts, I must also hope that those who differ from the theoretic view, as to the ultimate destination of the amyloid substance in the animal economy, which I advance in the following pages, will, at least, remember that I do not put forward my view with dogmatism, but as one keenly alive to the difficulties and delicacy of the question at issue.

When criticising the view which I advance, as to the ultimate destination of the animal dextrine formed by the liver, some persons may still be inclined to cling to the notion that this matter is destined solely for the maintenance of animal heat, and that it is consumed in the respiratory process. To ask such persons to consider and reflect upon the facts connected with the disappearance of the amyloid substance from the tissues of the fœtus before birth, is the best reply that can be given to this objection.

It may also be said, that the researches of Alexander Schmidt and others make it probable that fibrine may be present in the blood of the hepatic veins, but in a condition in which it does not coagulate; and if so, an important link is wanting in the chain of reasoning. It is, however, quite certain that, whatever names may be given to the materials which give rise to what is recognised as coagulated fibrine, some part, at least, of these materials has vanished from the blood which is leaving the liver; and that, therefore, in passing through the liver, either a destruction of fibrine has taken place, or something equivalent thereto. Globulin, or a closely similar substance, is certainly present in considerable quantity in the blood of the hepatic veins; and it has—to use the language of Schmidt—marked *fibrino-plastic* action in producing coagulation of hydrocele fluid, etc. If, therefore, it does not give rise to coagulation in the blood coming from the liver, it is because the *fibrino-genous* elements are wanting in this liquid.

Again, it may be argued that, if the theory put forward in these pages be correct, an animal would live for an indefinite period without receiving any *nitrogenous* food, inasmuch as the nitrogen in the system is constantly being used over and over again by the liver, in the reconstruction of a new formative material; and that, accordingly, an animal should be able to live exclusively on non-nitrogenous food. This, although not wholly true, yet is so to some extent. The effete nitrogen thrown out of the system by the kidneys, after it has done its work, must be replaced from without in order that life may be maintained. But there is no doubt that the amount of urea eliminated becomes diminished when a non-nitrogenous diet is administered; it is equally certain that animals which, when fed on gelatine, live only a few days, will live ten times as long if fed on starch and sugar exclusively.

Physiologists who may feel inclined to reproach me for not having made more definite and precise observations on the proteic compound, which is mentioned as being found in the blood of the hepatic veins more abundantly than in any other blood, will, I hope, remember, "that the land of protein bodies is a land full of undefined shapes, which only here and there present a well-marked form;" that our knowledge of these bodies is indeed most imperfect; and that their investigation constitutes a series of problems among the most abstruse to be met with in the whole range of animal chemistry.

14, Lower Pembroke-street,
Dublin, February, 1865.

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OBSERVATIONS

ON

THE FUNCTIONS OF THE LIVER.

INTRODUCTION.

In March, 1857, Professor Bernard announced the important discovery of a material formed by the liver, closely resembling starch, or rather dextrine of vegetable origin, and like it readily changed into sugar in the presence of ferments. The discovery of this amyloid substance did not alter his views concerning the formation of sugar in the liver, but rather served to place his so-called "glycogenic theory" on a more secure basis than before. Nevertheless, the researches of Dr. Pavy,* to say the least, cast great doubt on the correctness of this supposition, while the beautiful observations of Charles Rouget† and of Bernard‡ himself, on the fœtal tissues, tend to show that the amyloid substance (under certain circumstances) serves a very different end from that of being thus transformed into sugar.

Some years ago, after a careful repetition of the experiments of Dr. Pavy, I was led to agree§ with the conclusion arrived at

* Philosophical Transactions, 1860, p. 595.

† Journal de la Physiologie de l'Homme et des Animaux, tom. 2, pp. 83 and 308.

‡ Ibid. tom. 2, p. 326.

§ On the Physiology of Diabetic Sugar in the Animal Economy, "Dublin Quarterly Journal of Medical Science," August, 1859.

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by him, that the liver is not endowed with the power of converting its amyloid substance into sugar during life and health. If, therefore, not transformed into sugar, the question naturally arises, What becomes of it? What is its ultimate destination in the animal economy? It is my chief object in the following memoir to attempt to answer this question.

I have elsewhere* stated, that although the question must be admitted to be one of the greatest delicacy, yet that it appears to me, on the whole, there is evidence that the amyloid substance met with in the liver is, as it were, on its way upwards towards the more exalted or complex immediate animal principles, and that its conversion into sugar is not its normal destination; that the process of healthy assimilation tends, if the expression may be used, to promote it from the the rank of ternary (hydrocarbonous) to that of quaternary (azotised) compounds, and that its conversion into sugar is, therefore, a deviation from this progressive course—a *dissimilative* instead of an assimilative process. In order to establish this view it became necessary—

First.—To re-examine, with precision, the facts which have induced Pavy to conclude that the amyloid substance of the liver is not transformed into sugar during life.

Secondly.—To investigate the chemical and physiological relations of the amyloid substance, not only of the liver but of other organs and tissues, and test the very interesting results which are for the most part due to M. Charles Rouget.

Thirdly.—To compare the portal and hepatic blood with each other, and with arterial and venous blood derived from other sources, and consider the relations to each other of the different functions performed by the liver. For if it be true, as Lehmann, Brown-Séquard, and others have asserted, that the fibrine and much of the albumen of the portal blood vanish in the liver, and that, at the same time that it destroys these azotised compounds, it forms its non-azotised amyloid substance and excretes bile, containing so little nitrogen that it need hardly be taken into account; are we not, from the consideration of these functions, led to infer that the nitrogen, which leaves the liver by no other outlet, may go forth in the hepatic

* On the Formation of Sugar and Amyloid Substance in the Animal Economy. Proceedings of Royal Irish Academy, February, 1860.

blood, in union with the amyloid substance thus changed into a new azotised principle? That thus the liver is a great blood-making organ, in which there is constantly going on a reconstruction of certain ingredients of the blood; that in it the fibrine, &c., which has done its work, is disintegrated, the hydro-carbons of the bile abstracted, and the nitrogen combined with the amyloid substance, which, instead of being normally changed into sugar, emerges from the liver as a constituent principle of the protoplasma, from the bosom of which (to use the words of Bernard with reference to the fœtal tissues) organic evolution is to be accomplished.

§ 1.

EXAMINATION OF THE FACTS TENDING TO PROVE THAT THE AMYLOID SUBSTANCE OF THE LIVER IS NOT TRANSFORMED INTO SUGAR DURING LIFE AND HEALTH.

It may at first appear unnecessary to do more than state my conviction of the soundness of the views, and the accuracy of the experimental investigations of Dr. Pavy; but, as no other physiologist has as yet fully corroborated his assertions, and as it is essential to my purpose to show the grounds upon which I have been led to concur with him in thinking that, during life and health, little, if any, of the amyloid substance of the liver is converted into sugar, I must be allowed to offer my testimony on this subject at some length.

In obtaining blood by the use of the catheter from the right side of the heart, I had become dexterous from frequent performance of the operation. In less than two minutes, with little struggling on the part of the animal, and but slight apparent injury to it, between two and three ounces of blood can be abstracted. The end of the catheter was found, in making previous trials on dead animals, sometimes to pass the heart, and go down the ascending cava. In order to be certain that one is not abstracting blood from below the point at which the vessels of the liver enter the ascending cava, it is necessary, therefore, to make accurate measurement beforehand, so as not to pass the instrument too far in. When the catheter has been successfully introduced from one of the veins in the neck into the right side of the heart, it is gently moved up and down while the blood is being drawn into the pipette attached to it; by this manœuvre the operation is facilitated, and the blood is not prevented from entering the catheter by the lining membrane of the heart or vein being sucked against the eye of the instrument. The blood thus obtained from the right side of the heart was treated with three or four times its bulk of proof spirit, which was filtered off, the residue being repeatedly washed with additional spirit; the filtered spirit was evaporated to dryness on a water bath, and the dry residue treated with distilled water, which was tested by Fehling's solution for the presence of sugar. The result was, that in twelve experiments made on dogs, which for some weeks before had been fed exclusively on meat, traces of sugar were found in five; no sugar was discoverable in the blood of the remaining seven. None of these animals were killed at the time; but there is no reasonable doubt but that, as they were healthy animals, more or less of the amyloid substance existed in the livers of every one of them, and that, probably, the traces of sugar in five were due to the dislodgement of some of it, in consequence of unavoidable struggling, and its transformation into sugar in the blood.

In experimenting on the tissue of the liver of warm-blooded animals immediately after death by pithing, no matter what rapidity, precision, and care are exercised, it is not possible to obtain results which enable one to state that absolutely no sugar is present; but the quantity, under certain circumstances, is so small as to be quite incapable of being estimated. When we consider the extraordinary delicacy of the tests for sugar now in use, such a result is what must be naturally expected, when it is recollected that the temperature of the pithed animal is highly favourable to the prompt change of the amyloid substance into sugar, and that in boiled starch the presence of saliva is in a few seconds able to give rise to sugar transformation.

In repeating Dr. Pavy's experiments on the tissue of the liver by freezing it or boiling it, with the least possible loss of time, after death I have preferred to operate on cats rather than rabbits, because more or less sugar almost invariably pervades the tissues, or is discoverable in both the venous and arterial blood of these vegetable feeders. Cats, therefore, for some time fed on meat, and killed during full digestion, and when a considerable quantity of amyloid substance exists in the liver, offer a fairer prospect of a truthful result as regards the transformation of this material into sugar. The following are the details of two experiments, out of several, giving as nearly as possible similar results :—

A large, healthy female cat, for some days before fed exclusively on flesh meat, was pithed three and a-half hours after her last meal. At once a portion of the liver was cut off and thrown into a quart of boiling distilled water. After being boiled for fifteen minutes, it was taken out and weighed, and returned again to the same water. An equal weight (exactly 500 grains) of that portion of the liver which had remained in the animal was then (*i.e.*, after the lapse of twenty minutes) likewise placed in a quart of cold distilled water and boiled. Each was boiled down to one-half. The portions of liver in each were bruised to pulp, and the boiling of each was then renewed, and finally each was evaporated to dryness on a water-bath. Each was treated with four ounces of proof spirit, which was filtered off, and the residue washed with additional spirit. The spirit was evaporated on a water-bath, and the dry residues treated with equal quantities of distilled water, and filtered so as to give a solution sufficiently clear for the application of the cupro-potassic test.

RESULT

500 grains of liver, boiled immediately after death, gave an indication of sugar so slight as to be almost inappreciable, and incapable of being estimated.

500 grains of liver allowed to remain in the body of the animal for 20 minutes after death, contained 12·5 grains of glucose.

A large, healthy male cat, for some days previously fed exclusively on meat, was pithed between three and four hours after his last meal. At once a portion of the liver was cut off, and thrown into a capsule, previously immersed in a freezing mixture of extreme coldness. This portion of liver was immediately bruised with a pestle, already reduced to a very low temperature by immersion in the freezing mixture. In a very few seconds the whole was frozen quite hard on the sides and bottom of the capsule. It was then treated with two ounces of proof spirit, which, after the lapse of two hours, was filtered off, and which passed through the filter almost perfectly clear: this, with the washings of the residue, was evaporated to dryness on a water bath, and the residue treated with distilled water, so as to obtain a solution clear enough for the use of the cupro-potassic test.

A portion of the liver, which had been allowed to remain in the body of the animal for twenty minutes, and which was, judging by the eye, about the same size as that at once cut out and frozen, was bruised up with two ounces of proof spirit, which, being filtered off, was evaporated to dryness, and the residue heated with distilled water, so as to obtain a solution suitable for testing.

RESULT.

The portion of liver frozen immediately after death, gave an indication of sugar barely perceptible.	The portion of liver allowed to remain 20 minutes in the body, contained 3·8 grains of glucose.

It appears, then, that these experiments give results very closely agreeing with those of Dr. Pavy, but there are others even more strongly corroborative of his views. Thus, if a healthy, well-fed hedgehog be gradually reduced in temperature, by having ice placed near it until it becomes torpid, and the temperature then further reduced until, by degrees, the entire animal is slowly frozen into a solid mass, the liver then taken from the body, and treated as above described, does not give the slightest evidence of the existence of sugar. By this device it is possible, as it were, to steal so gently upon life, that at the moment of its departure the amount of cold is sufficiently great to prevent any of the amyloid substance, which is by no means wanting in the livers of these animals, being converted into sugar. The fact that no sugar is found when this experiment is carefully performed, is, perhaps, the most conclusive evidence which can be offered, that, during life and health, the liver is not normally employed in effecting this transformation.

Although varying a little at different seasons of the year, yet it may be stated that, on an average, the liver of a full-grown frog will yield about 1·5 grains of amyloid substance. Now, if, as in the case of the hedgehog, a frog be gradually reduced to a low temperature, and ultimately quite frozen, the liver then taken from the body does not give the slightest trace of sugar. It is necessary, however, in order to succeed in this experiment, that the lowering of temperature should be very gradually effected.*

From the foregoing and many other less important modifications of the ingenious and exhaustive experiments of Dr. Pavy, I feel justified in stating my conviction of the correctness of his views. Although it must be admitted, that causes, apparently very trifling, will give rise to saccharine transformation, and, as a matter of experiment, the subject is one beset with difficulties, and requiring great nicety and precision of manipulation; yet, on the whole, I believe, there is sufficient evidence to lead to the conclusion, that, under circumstances of perfect health, transformation into glucose is not the normal destination of the amyloid substance formed so abundantly in the liver.

§ 2.

OF THE PHYSIOLOGICAL RELATIONS OF AMYLOID SUBSTANCE OF ANIMAL ORIGIN.

In entering upon the investigation of the chemical and physiological relations of the amyloid substance of animal origin, the subject must be studied—

* The following statement shows the necessity of attending to these instructions. I requested the Reverend Professor Haughton, F.T.C.D., to let me have the benefit of his critical acuteness while repeating in his presence some of these experiments. A large frog was placed in a tin canister, which was immersed in a freezing mixture of ice and salt. In less than twenty minutes the animal was frozen quite hard, the liver removed, broken up in a mortar, and treated in the manner described above. To my surprise, Professor Haughton discovered very unmistakeable evidence of sugar, amounting to more than one-tenth of a grain. It occurred to me that the rapid freezing was the cause of a result quite different from what I had before repeatedly obtained when the frogs had been gradually cooled by placing pieces of ice near them at first, until they gradually became torpid. The extreme cold, coming rapidly through the tin canister, had made the frog jump about violently at first; the struggling thus produced probably dislodged some amyloid substance, which was then transformed into sugar. Upon repeating the experiment, and making use of cold so gradually as first to render the animals torpid, the results, as above stated, were obtained. Thus in two frogs, there was no evidence whatever of the presence of sugar, while the two livers yielded 2·40 grains of amyloid substance.

First.—As regards the formation of this substance by the liver.

Second.—With reference to its existence in the placenta and other organs and tissues (especially those in process of development); and,

Third.—Under its pathological aspect: for, although in some particulars differing from each other, the consideration of the distinct varieties of amyloid substance throws some light on the probable ultimate destination of the animal dextrine formed by the liver during life.

To avoid confusion, it is well to state that the term, "amyloid substance," is used as a generic term applied to animal substances having special characters; in some varieties presenting the characteristics of starch, or, rather, dextrine of vegetable origin, yet in others giving unquestionable indications of the presence of nitrogen. The species of amyloid substance discovered by Bernard in the liver, and now known to exist normally in other tissues and organs belongs to the former group, and being free from the intimate admixture of azotized matters, is consequently distinct from the amyloid substance discovered by Virchow, which, although in histological characters, analagous to cellulose and starch, yet, as met with in the prostate gland, spleen, kidney, choroid plexus, &c., is, without doubt, in union more or less complete with nitrogen. For the sake of clearness, I have elsewhere* proposed to indicate the first of these varieties as *the amyloid substance of Bernard*, or being, evidently, very closely related to vegetable starch, *of the first species;* the latter, as amyloid substance of *Virchow*, or *of the second species.*

The amyloid substance of Bernard—which variety, for the most part, concerns us at present—is now so well known, that it is only necessary, very briefly, to recapitulate its chief features, and to mention the process by which it has been obtained in the following researches. According to Pelouze, it is a ternary compound isomeric with dried grape sugar; by the action of fuming nitric acid he has transformed it into xyloidin, and he gives for it the formula C_{12}, H_{12}, O_{12}. Professor Apjohn, of Trinity College, Dublin, has been so good as to make an

* Proceedings of Royal Irish Academy, February 13th, 1860.

ultimate analysis for me of a specimen prepared by myself from the livers of rabbits. He finds that in 100 parts it has the following composition :—

Carbon, ...	43·78
Hydrogen, ...	6·32
Nitrogen, ...	·62
Oxygen, ...	49·28
	100·

"Neglecting," he says, "the trace of nitrogen as an accidental impurity, I find that these results are very accurately represented by the formula C_{12}, H_{10}, O_{10}, which is that representing the constitution of starch and dextrine also."

It is a neutral, whitish, inodorous, insipid matter, soluble in water, insoluble in alcohol and strong acetic acid. In its reactions it is identical with vegetable dextrine.

In the presence of saliva and other animal ferments it is converted into sugar, which ferments on the addition of yeast, and reduces the cupro-potassic solution ; iodine in contact with it produces a peculiar brown coloration, more or less intense, disappearing on the application of heat, and re-appearing when the fluid cools below 80°.

It was first obtained by Bernard,* by treating the boilings of the liver with four or five times its volume of absolute alcohol, and subsequently freeing the precipitate thus formed from azotized matters by boiling it for some time in a concentrated solution of caustic potash. This method is objectionable. The acetic acid process, although not economical, is preferable, and, indeed, is invaluable as a test for the presence of the substance in question in the various tissues of the organism.

The organ or tissue to be examined is boiled in a small quantity of distilled water ; the whole is then bruised in a mortar with animal charcoal, thrown on a filter, and some drops allowed to fall into glacial acetic acid. If amyloid substance

* It is right to state, that Hensen, if not prior to, at least independently of Bernard, isolated this substance. In December, 1856, he exhibited some of it before the Naturwissenschaftliche Gesellschaft at Wurzburg ; and on the 1st of April, 1857, in Virchow and Hoppe's Pathological Institute. But the French physiologist has the advantage of having first published an account of it, and of the manner in which it was obtained.

of the first species is present, it forms a more or less abundant white flaky precipitate.

Gelatine and casein are not completely arrested upon the filter by the animal charcoal, but the former is soluble in acetic acid, and the latter, although at first precipitated, is at once re-dissolved by the glacial acid. These substances, therefore, do not interfere where acetic acid is used, but the contrary is the case with alcohol.

If the tissues, while still raw, be pounded in a mortar with animal charcoal, the albuminoid materials are more completely retained by it upon the filter; but one never can feel satisfied that absolutely no gelatine passes through when fibrous and muscular structures are being examined.

For this reason the acetic acid process seems less subject to error than the method in which alcohol and potash are used; and accordingly, in the following experiments, the former has been the process generally adopted. As, however, the decoction of a liver ordinarily contains no very excessive amount of albuminoid matter, the quantity of amyloid substance in a given weight of liver tissue may be very accurately determined by treating the boilings, when filtered, with five or six times their volume of proof spirit, collecting the precipitate on a filter, drying and weighing it.

For the detection of amyloid substance in certain tissues, the aid of the microscope is indispensable; with the help of acidulated tincture of iodine (equal parts of tincture of iodine and acetic acid), it may be discovered with perfect certainty in the cells of tissues: the brown colour arising from the contact with iodine, may be dispelled by gently heating the slide on which the object rests, and when the colour is seen to return again on cooling, no doubt need any longer be entertained as to the presence of the amyloid substance of Bernard.

In dealing with the fœtal organs and tissues, owing to the large amount of gelatine extracted by boiling them, it is necessary to be very careful. The acetic acid process answers very well for this purpose, but the use of a strong solution of caustic potash in alcohol, as suggested by Bernard, will also be found very useful. It is thus prepared: a considerable quantity of pure caustic potash is dissolved in alcohol. If kept for some time it becomes sherry-coloured, and gradually even deeper; but it should be made only when required, and used before becoming discoloured. A portion of the tissue to be examined—suppose a mass of muscle of a fœtus—is placed in ten or twelve times its bulk of this solution; if shaken from time to time, in twenty-four hours all the albuminoid matter will be dissolved away. The amyloid substance remaining undissolved may be repeatedly washed with alcohol, and thus obtained in a tolerably pure state. At all events, the relative amount of amyloid substance in different fœtal tissues, may thus be determined with great nicety.

Dr. Pavy has stated,* that the power of diffusion of the amyloid substance of Bernard is so low, that it does not pass through animal membranes at an ordinary pressure. In fact, it belongs to the class of *colloid* bodies, and does not pass through Graham's dialyser, by which apparatus it may be separated from sugar and other crystallisable impurities.

(A) *On the Formation by the Liver of the Amyloid Substance of Bernard.*

The independent testimony of many physiologists shows that the liver of an animal fed exclusively on meat, can form within itself abundantly amyloid substance. The ordinary beef and mutton of our markets contain no material of the nature of starch or sugar;† nevertheless, animals fed exclusively on such food form amyloid substance abundantly, even more so than graminivorous animals do. Thus, a large dog, which had been restricted to flesh diet, and the liver of which weighed 10·5 ounces, being killed during full digestion, yielded 6·5 per cent. of amyloid substance; while a sheep,‡

* Proceedings of Royal Society, vol. x. p. 530.

† See experiments detailed, in Proceedings of Royal Irish Academy, July, 1860.

‡ It is striking how small an amount of amyloid substance is found in the sheep's liver to be obtained in the market. At first this appeared to me to be owing partly to the fact, that the livers are not very fresh, and partly to the fact that the sheep are not fed for some time before being killed. To obtain a correct result, I caused sheep to be driven in from pasture, food was offered the next day, so that being killed some time after, the liver might be obtained during full digestion. Sheep, however, will not feed in captivity, and when separated from their fellows; it was necessary, therefore, to restore the animals to their pasture and companions, and when they had filled themselves to recapture them. In this way

also killed during full digestion, and the liver of which weighed 35·5 ounces, gave little more than one per cent. of amyloid substance.

This result becomes even more striking when it is remembered, that, in relation to the weight of the animal itself, the liver of graminivorous animals is small as compared with that of the carnivora.

In animals living on their ordinary diet, and supposed to be in health, not only does the weight of the liver, as compared with that of the whole body, vary considerably, but also the percentage of amyloid substance found in the liver varies to a great degree. The following table shows what may be assumed as the normal average relation existing between the liver and the body, as well as the normal percentage of amyloid substance met with in the livers of different animals :—

* Relative weight of liver to the entire body of the animal.	Percentage of amyloid substance found in the liver.
As 1 to 30 in dogs - - -	4 5 grains per cent.
1 to 19 in cats - - -	1·5 ,,
1 to 35 in rabbits - - -	3 7 ,,
1 to 44 in pigeons - - -	2·5 ,,
1 to 21 in guinea pigs - - -	1·4 ,,
1 to 26 in rats - - -	2·5 ,,
1 to 27 in hedgehogs - - -	1·5 ,,

In order to make an accurate comparison between the size of the liver, as compared to that of the entire animal, in meat-eating and vegetable-eating animals, cats and rabbits were selected, as being easily obtained, about the same size, and also on account of their feeding and thriving well in captivity. From a considerable number of experiments it may be stated, in general terms (and the fact is an important one, to be subsequently alluded to), that the livers of healthy cats fed on meat are nearly double the weight of the livers of rabbits, the animals being of the same size, and the experiment being made

only is it possible to obtain a correct comparison so far as being killed during digestion is concerned, which is of the first importance as regards these investigations.

* This table is made on average taken from six examinations of each animal. All were on their ordinary or natural diet, and in each the body was weighed after the removal of the liver. The gall bladder was separated from the liver before it was weighed, and in all cases digestion was going on with activity when the animals were killed.

at the time of full digestion. But the livers of the meat-eaters are not nearly so rich in amyloid substance, so that, even allowing for the much greater bulk, the liver of a large, well-fed cat will not yield more than two-thirds as much of this material as the liver of a healthy rabbit fed on carrots, bread, and parsley.

The amyloid substance found in the tissue of the liver is met with in greatest abundance about six hours after a full meal. From this time, if the animals are not fed, it diminishes gradually and steadily, and in starved animals it has wholly disappeared.

Six large, healthy rabbits were fed on carrots, white bread, and parsley early in the morning. At eight o'clock, A.M., all food was taken from them, and in four hours from that time one was killed, and the amount of amyloid substance in the liver determined, and so on, one every four hours. The result was as follows :—

	Quantity of amyloid in the entire liver.
1st rabbit, 4 hours after feeding - -	32 grains.
2nd ,, 8 ,, ,, - -	43½ ,,
3rd ,, 12 ,, ,, - -	27½ ,,
4th ,, 16 ,, ,, - -	23 ,,
5th ,, 20 ,, ,, - -	19 ,,
6th ,, 24 ,, ,, - -	14½ ,,

After some days of abstinence, it is no longer possible to discover in the liver any more than a trace of amyloid substance.

This disappearance of the amyloid substance from the liver during abstinence, is not retarded by heat nor accelerated by cold. Six pigeons were fed for some days on a mixture of starch, potatoes, and brown sugar. Two of them were killed five hours after their last meal. The liver of one contained 13½ grains of amyloid substance; that of the other 15 grains. Two more were not fed at all during three days, all which time they were kept in an oven heated to from 110° to 120°. The liver of each of these yielded hardly an appreciable quantity of amyloid substance. The two remaining pigeons were also not fed during three days, but were kept in a place where the thermometer during the day did not rise higher than 60°. The liver of one of these gave 1¼ gr. of amyloid substance; that of the other, an inappreciably small quantity.

Dr. Pavy has shown how great is the effect of diet containing much starch and sugar on the amount of amyloid substance formed by the liver. It is surprising, and, indeed, almost incredible, to what a degree and with what rapidity the liver may be increased or diminished in bulk by the administration of particular kinds of diet. So far as this is due to an increased or diminished quantity of the amyloid substance stored up in the liver, the following table will show :—

Average quantity of amyloid substance found in the entire liver of animals fed for some days on the following materials.

	On a diet consisting almost entirely of sugar and starch.	On a diet of fat.	On diet of gluten bread.	On a diet of gelatine.
Dogs, . .	980 grains.	hardly a trace	125 grains.	none.
Rats, . .	7 ,,	ditto.	3 ,,	none.
Pigeons, .	25½ ,,		1 ,,	
Rabbits, .	45 ,,		8½ ,,	

Bernard* is in error in asserting that the animal organism can prepare amyloid substance from gelatine. It is certain that the livers of dogs and rats fed on gelatine give the same results exactly as the livers of these animals when no food at all is given to them ; and the same is true when they are fed on fat.

From fibrine of the blood, from flesh meat, and from gluten of wheat, the liver is able to make amyloid substance. But it seems to be capable of secreting it with much greater facility, and in much greater abundance, when saccharine matters are introduced into the stomach. The formation of it from nitrogenous materials appears to be a more difficult matter, and hence it is probably that, if one may use the expression, as the liver has more up-hill work in meat-eaters, it attains proportionally a much greater size than in vegetable feeders, without, in general, making so large a quantity of amyloid substance.

It is not necessary, however, that saccharine and amylaceous

* L'Union Medicale, No. 35, p. 551, 1859.

substances should be present in the food to insure the formation of amyloid substance by the liver. Strictly carnivorous animals seem to form it even more easily from meat than vegetable food, if the latter can be introduced. Cats are sometimes very fond of asparagus, and if fed on this vegetable, it is found that the liver gets small, and forms but little amyloid substance. Two cats of the same size, age, colour, and sex, were fed, one on flesh meat, the other on asparagus, for eight days; they were then killed. The liver of that fed on meat weighed 1230 grains, and yielded 17 grains of amyloid substance, while the liver of the other weighed but 630 grains, and gave but 3 grains of amyloid substance. Thus, in the course of a few days, the liver of the one was found to weigh little more than one-half as much as that of the cat fed on the diet which it is natural to presume was best fitted for its nourishment, although less rich in saccharine materials.

(B) *Of the Existence of the Amyloid Substance of Bernard in the Placenta and Amnion, &c.*

The cells of the placenta contain, during the earlier stages of embryonic life, animal dextrine, having characters identical with those of the amyloid substance of the liver. Its presence may be demonstrated under the microscope with great facility. By the use of the acidulated tincture of iodine, the peculiar brown colour may be produced, which will be found to disappear on the application of gentle heat, and return again on cooling. It may be obtained, however, from the placenta by boiling water, just as it can be from the liver, and in sufficient quantity to be submitted to chemical examination, converted into sugar, fermented, &c. It is apparently equally abundant in the placenta of cats and of rabbits, and in each it is found in larger quantity at a time when the growth of the fœtus is progressing rapidly. The placentæ taken from a single rabbit, four weeks pregnant, gave five grains; those from a cat, about five weeks pregnant, six grains.* Shortly before the birth of the young, it has either totally or almost totally disappeared from the placenta.

* This was estimated by the alcoholic solution of potash, and must, therefore, be considered only an approximate result.

Bernard* discovered the presence of amyloid substance in the placenta of rabbits, guinea pigs, &c., the epithelial cells of which, at an early period of their development, are filled with it. The cells containing it appeared to him to be situated principally between the maternal and the fœtal portions of the placenta. He also made the very interesting observation, that the multiple placentulæ of the ruminants do not contain any amyloid substance, but that in this class of animals this substánce is found in certàin cells of the amnion, which, in some ruminants, are collected into masses on the fœtal surface of this membrane. These masses which, true to his glycogenic theory, he calls "les plaques hepatiques, de l'amnios," in the cow are studded in great numbers over the inner surface of the amniotic membrane. To the naked eye they look like drops of wax of various sizes, from that of a millet seed to that of a split pea, or larger, sticking over the amnion, but not evenly distributed over it. These amyloid patches of the amnion have not the appearance of being glandular bodies, but consist of large epithelial scales, filled with amyloid substance. In the sheep, patches of the same kind as in the cow do not exist. In this animal the amyloid substance exists in the epithelial cells which line the sac of the amnion, and in the papillæ found on this membrane, where it covers the funis and elsewhere. A careful examination of these structures, made by any one whose mind is not already pre-occupied by the theory of glycogenesis, will, I conceive, lead the observer to admit that the view of M. Charles Rouget† is really the correct one ; that the papillæ, the patches, the warty growths of the amnion, are nothing else than productions by which the amnion tends to show the identity of its nature with that of the skin which it represents, and with which it is continuous. The presence, however, in the amnion or the placenta of epithelial cells, containing amyloid substance, is a fact quite secondary to the general fact, that this substance enters largely into the constitution of most of the tissues of the embryo. Its existence does not indicate a new function of an organ doing for a time the duty of the liver, but it indicates a new fact with regard to the develop-

* Journal de Physiologie, tom. ii. p. 31.
† Journal de Physiologie, tom. ii. p. 321.

ment of certain structures, and a new property of tissue. The production of sugar in the liquids of the allantois and of the amnion, is not the object, but the consequence of the presence of this substance in the fœtal structures. This sugar results from a *disassimilation* of some of the amyloid substance, which exists in the tissues of the fœtus for a very different purpose than glycogenesis.

Of the Amyloid Substance met with in the Tissues of the Fœtus.

During embryonic life, a great part of the fœtal tissues are found to be so impregnated with amyloid substance, that, in truth, it would appear to be the formative material from which these tissues are evolved, and would seem related to their growth and development, as starch is to the growth and development of vegetables.

The amyloid substance met with in the fœtal tissues, is in chemical composition identical with that found in the liver. Absolutely pure specimens, prepared from each of these sources, are represented by the formula C_{12}, H_{10}, O_{10}. In a former memoir, speaking with reference to its optical properties, it was stated, on the authority of French observers, that amyloid substance of animal origin, like vegetable dextrine, causes the plane of polarization to deviate to the right. I must now confess that I have not been able to verify this assertion. It is not possible by any means that I have been able to devise, to obtain a solution of this substance so transparent as to admit of its being submitted to examination in the saccharometer. If a portion of the liver of an adult animal, or of the muscular tissue or lung of a fœtus, be pounded to a pulp in a mortar with silver sand, and the whole afterwards mixed into a paste with animal charcoal, and allowed to stand for some hours, and then treated with boiling distilled water, and filtered, the liquid thus obtained is too turbid to permit of its rotatory power as regards polarized light being investigated. So small a quantity as half a grain of pure amyloid substance, dissolved in an ounce of distilled water, produces in the solution a peculiar opalescent appearance.

I have proved by experiment that this is not due to fluorescence, but to the fact that the amyloid substance has its particles merely in a state of suspension, not of true solution. No trace of it will pass through a dialyser without the exercise of pressure, and the liquid thus obtained is not sufficiently translucent for examination by polarized light.

M. Charles Rouget and Professor Claude Bernard have examined the tissues of the fœtus microscopically, so as to determine the presence in several of them of amyloid substance in abundance; but neither of these observers has attempted to show by chemical investigation at what period of development each of the tissues containing it is found to have it entering most largely into its composition. It must be remembered, that the acidulated tincture of iodine is a test of such delicacy for this substance, that it produces its characteristic reaction, even when the quantity of amyloid substance present is very minute : hence, judging from the use of this test under the microscope, one is apt to suppose that the amount present is greater than it really is ;* or rather, that it is equally abundant in tissues which, in reality, contain it in widely different quantities. It was possibly owing to this mode of examination, that Professor Bernard was led to suppose that this substance continues to exist in muscular tissue during the entire period of intra-uterine life, and that it does not disappear until after birth, when it does so under the influence of the respiratory and muscular movements.

I hope to be able to show, however, that the establishment of respiration has little to say to the disappearance of the amyloid substance from the tissues of the fœtus, and to prove that, in truth, certain azotised tissues are evolved from a nidus of amylaceous protoplasm, which, after a particular stage of growth, becomes less and less as each of these tissues approaches maturity, and that, when maturity is attained, the amyloid substance, which once formed so large an ingredient of the growing tissue, has gradually become changed, so as to be no longer discernible even before respiration has commenced.

M. Rouget has quite correctly pointed out the very early period at which the amyloid substance is found in the cartila-

* A tenth of a grain of amyloid substance may be readily detected in an ounce of water, by the action of acidulated tincture of iodine.

ginous tissue. It first shows itself in the cellules of this tissue ; in the embryo chick and lamb, at a very early stage of development, it may be demonstrated. It very soon, however, disappears from the cells of cartilage, and is too small in amount to be estimated at different stages of growth.

The epithelial cells of the skin are rich in amyloid substance at an early period ; the points where cells aggregate themselves together for the commencing development of a feather or a hair, show a great abundance of the amylaceous material. The horny appendages of the skin, the bill of the embryo chick, the claws, hoofs, &c., of other embryos, contain it in large quantity up to a particular period of development. The feet of a fœtal calf of about four months, were dried at a heat not exceeding 212°; 7 grs. of the horny structure were rasped off, and on examination yielded 1·3 grs. of amyloid substance. An exactly equal quantity from the feet of a nearly full grown fœtal calf, similarly treated, gave an amount of amyloid substance too minute to be estimated. It almost wholly disappears from the feathers when they become prominent on the surface ; and for hairs, the same may be said. If one of the large hairs from the eyebrow of a fœtal lamb, shortly before the time of birth, be examined, nothing more than a mere trace of amyloid substance can be detected, and that only in the cells of the bulb. If a number of embryo lambs, of various sizes, are placed side by side, and a drop of a weak acidulated solution of iodine be allowed to fall on a corresponding part of each, the peculiar brown stain produced will be seen to increase in intensity up to a certain point. After the appearance of the hair, the stain gradually diminishes in intensity, showing the lessening quantity of amyloid substance in the tissue. If the tails cut off from a series of fœtal lambs are placed in a vessel containing a very weak acidulated solution of iodine, it illustrates, by the colouring, very strikingly, the increasing abundance of the amyloid substance in the epidermic tissue, up to a particular period (that is, when the hair is fully formed), and then its gradual disappearance. The feet and hoofs, similarly treated, illustrate the same for the horny tissue of this part. From the time that the fœtus of the sheep is nine inches in length (at which period it seems to be at its maximum), the amyloid substance contained in the horny ·ure of the hoof gradually diminishes, until shortly before birth, when, even after prolonged boiling, no trace of amyloid substance can be extracted from it.

In the tissue of the lung of mammalian embryos, the amyloid substance is at one period present in immense quantity. After the watery part is driven off by evaporation, more than 50 per cent. of the dry residue is found to be nothing else than animal dextrine. As the organ approaches maturity, and the animal is about being born, but before it has yet drawn a single breath, the amyloid substance is found to be reduced to a very small quantity indeed, and in some instances to have absolutely disappeared. The following table shows the progress of this change in the lung of the embryo of the sheep, and, I believe, very closely represents the corresponding amount of change which takes place in the lung tissue of other embryos which I have examined, viz., of the rabbit, cat, dog, cow, rat, guinea pig:—

Size and Condition of the Embryo.	Amount of amyloid substance contained in 20 grains of the perfectly fresh lung tissue.
1st—Not quite 6 inches long, without any vestige of hair,	1·9 gr.
2nd—7 inches long, a trace of hair on the lip,	2·55 grs.
3rd—10 inches long, delicate hairs about the head,	2·3 grs.
4th—15 inches long, covered with delicate hairs,	3·45 grs.
5th—16½ inches long, well covered with fine hair,	2·2 grs.
6th—Nearly 20 inches long, quite thickly covered with wool, and evidently very near the time of birth,	A quantity too small to be estimated.

It would be no easy matter to attempt to indicate precisely the condition of development of the embryo, at which the maximum amount of amyloid substance is to be found in the tissue of voluntary muscle; in embryos of apparently the same age and condition of development, it is found to vary a good deal in amount. But this much may be asserted positively, that, for some time before birth, it has much diminished in quantity in this tissue, although generally existing in it in notable amount up to and after birth.

After repeated examinations of various embryos, I believe I may state, that the following table, made from examinations of the voluntary muscular tissue of fœtal lambs, correctly represents the average quantity of animal dextrine found in this tissue at various periods of its growth :—

Size and Condition of the Embryo.	Quantity of fresh muscular tissue examined.	Weight of the foregoing when thoroughly dried.	Amount of amyloid substance in foregoing.
1st.—4 inches long,	30 grs.	1·7 gr.	·1 gr.
2nd.—7 inches long, hair on lip,	60 grs.	5·5 grs.	·5 gr.
3rd.—10 inches long, hair on head very fine,	60 grs.	6·2 grs.	1·1 gr.
4th.—15 inches long, covered with delicate hair,	60 grs.	7·5 grs.	2 grs.
5th.—16½ inches long, well covered with fine hair,	60 grs.	7·8 grs.	2·1 grs.
6th.—Nearly 20 inches long, and almost about to be born,	60 grs.	9·5 grs.	1·4 gr.

It appears, therefore, that although it exists in a less proportion than at an earlier period, there is in muscular tissue, at the period of birth, a considerable quantity of amyloid substance, and this does not disappear altogether for some time—in lambs occasionally not for some weeks after birth. The tissue of voluntary muscle cannot be considered to have attained maturity at the time of birth ; it has, as yet, hardly been brought into action, and has barely been yet called upon to exercise its functions. There is, however, a muscular organ, the tissue of which commences, of necessity, the active exercise of its functions at an earlier period than that of voluntary muscle ; the muscular structure of the heart, so far as its functions and activity are concerned, attains maturity earlier than other muscular tissue. The relation which the amyloid substance bears to it is, therefore, of much interest. In all embryos, without exception, which I have had an opportunity of examining at a time when they were closely approaching the period of birth, there has been no more than a trace of amyloid substance remaining in the muscular structure of the heart. The following table, drawn up from examination of the heart of the embyro of the sheep, closely represents the corresponding state in other embryos:—

Size and Condition of the Embryo.	Weight of the muscular tissue of the heart examined.	Amount of amyloid substance in foregoing.
1st.—7 inches long,	20 grs.	1·52 gr.
2nd.—10 inches long,	20 grs.	1·60 gr.
3rd.—15 inches long,	20 grs.	1·76 gr.
4th.—20 inches long, just before birth,	20 grs.	A trace too small to weigh.

The liver, which is the organ destined to form the amyloid matter during adult life, naturally has an increase of this material going on in its tissue up to and after birth. It does not make its appearance in the liver until the embryo is already well-advanced in its development; it then is found gradually and very slowly to increase in amount, but even at the time of birth is present in comparatively small quantity (·2 per cent. in the liver of a lamb 20 inches long). It may be asked then, What is the function of this material during fœtal life? It can, at least, be said that it does not change into sugar, neither does it give rise to fat. Does it not seem to be a formative material, which gradually becoming united with nitrogen, gives origin to the azotised structures constituting each full-grown tissue?

Amyloid Substance is, under certain circumstances, found to exist in the Tissues of Adult Animals.

The rapidly-growing horn of a young stag was not found to contain any amyloid substance in the tissues, neither does it exist in the texture of the growing horn of the calf. It is not found in the hair bulbs of the adult, neither is it to be discovered as a formative material of the newly-formed muscular fibres of the uterus, when this organ is undergoing its remarkable reconstruction after delivery.

But, when adult animals are fed on food containing much starch and sugar, the muscular tissue becomes impregnated with dextrine, having all the characters of the amyloid substance of

Bernard. A pigeon was fed for six days on starch and sugar; the liver yielded 25·5 grains of amyloid substance, and 5 grains of an identical material were obtained from the muscles of the breast. As a general rule, it is not to be found in the flesh-meat of the markets, but I have met with it in the muscular tissue of the cod, the skate, and occasionally in rabbit's flesh, apparently as a normal ingredient. Sanson* has demonstrated its presence in the flesh of horses. It exists also in the flesh of hybernating animals, but not abundantly; and whether, in this case, it is merely an impregnation of the muscular tissue of the animal, with a material formed in excess by the liver, as when animals are fed on starch and sugar, or whether it is due to an arrest of the normal nutritive process of muscle, resulting from inactivity, it is not possible to determine with exactitude.

(C) *Of the Amyloid Substance met with in the Animal Organism as a Pathological Deposit.*

As pathological deposits, substances more or less resembling starch or dextrine, of vegetable origin, are met with in the animal organism in at least two forms which differ considerably from each other. One of these is a material closely similar to, if not absolutely identical with, that hitherto spoken of as the amyloid substance of Bernard. It is met with in muscles paralysed by pressure on, or division of the nerves which supply them, and has all the physical properties and reactions of the amyloid substance met with in the liver; it can be obtained almost, if not quite free from any admixture of nitrogen, and is readily changed into sugar capable of fermenting. In the other form the pathologist recognises the material known to give rise to the so-called amyloid degeneration of divers organs, by the formation in them of a deposit possessed of "all the structural, chemical, and optical qualities of starch, as it occurs in plants,"† but which cannot be obtained without

* Rapport lu a l'Academie de Medicine, par M. Poggiale.—Journal de la Physiologie, &c., tom. i. p. 549.

† Mr. Busk, in the Quarterly Journal of Microsp. Science, vol. i. p. 133.

a certain amount of nitrogen, either mechanically or chemically so closely united with it, as not to be capable of separation from it, and which cannot be changed into sugar capable of fermentation. This latter form I propose to designate as the amyloid substance of Virchow, by whom it was discovered, in 1854. The first of these is rarely met with as a pathological deposit; when it is, it is in muscles which are in a state of inaction from use, and in which in consequence the process of nutrition has been interfered with. The paralysed muscles of the hind limb of a guinea pig, on which a lateral half of the spinal cord had been divided some months before, were examined, and found to contain traces of a substance giving a brown colour with iodine, under the microscope. The same material was found in a more decided degree in the muscles of the hind limbs of rabbits paralysed by section of their nerves.

Virchow first discovered that variety of amyloid substance with which his name is connected, in the ventricles of the brain; the roundish, concentric bodies found in the ependyma ventriculorum, he found to become blue on the addition of iodine, and on the further addition of sulphuric acid, to change to a deep violet blue tint. This alone he considered sufficient to distinguish it from any other substance at that time known among the animal tissues; further research, however, has shown that, under the names of chitine and tunicine, substances closely resembling starch and cellulose form a constituent part of the animal organism. The amyloid substance of Virchow is met with in a peculiar degenerated condition of many organs—most frequently in the prostate gland, spleen, and kidneys. Virchow's views concerning it are well known; he conceives that the cells in which it is deposited become converted into a sort of corpora amylacea. That it is starch-like in its nature cannot be doubted, yet, having obtained it lately in considerable quantity,* I have completely failed in all attempts to convert it into sugar. If we try to extract it by boiling water from a tissue, which, examined under the microscope, gives with iodine the characteristic reactions, we cannot obtain any precipitate by letting the decoction drop into glacial acetic

* *Vide* case of "Amyloid Disease of the Kidney," Proceedings of the Pathological Society, Dublin, New Series, vol. i. part i. p. 51.

acid. When the tissue containing it is dissolved away by the alcoholic solution of caustic potash, and repeatedly and carefully washed with spirit (for the tissues of the adult turn nearly black from the action of the potash), we obtain at last a whitish amorphous powder, which, when burned on platina foil, still gives the odour of an azotised material, and which no plan that I could devise could change into sugar.

It would appear, then, that in this form of amylaceous deposit, we meet with a substance which, although intimately united with nitrogen, nevertheless has not altogether lost the characteristics of a hydro-carbonous compound; it may be regarded as the result of an interrupted process, which, if completed, would place the material in question among the azotised compounds of the animal organism.

Having arrived, by a repetition of Dr. Pavy's ingenious experiments, at the conclusion that the amyloid substance of the liver is not normally changed into glucose, and finding, on examination, the accuracy of the facts concerning the physiological relation of the amyloid substance to the fœtal and other tissues, discovered by M. Charles Rouget, and investigated by Bernard himself, the question presents itself: May it not be that the liver does for the adult what divers tissues do during the development of the fœtus? May not this great organ form, with the help of the amyloid substance secreted in its cells, a nitrogenous compound, just as the muscles of the fœtus convert the amyloid substance contained in them into the highly nitrogenous materials of muscular tissue? May not, in fact, the amyloid substance of the liver be the basis of an azotised protoplasma, forming a constituent of the blood of the adult animal, as the amyloid substance of muscle is the basis of the material from which the evolution of muscular tissue is accomplished?

A general consideration of the functions performed by the liver, leads one to answer these questions in the affirmative. For, if it be true that the blood which enters the liver is rich in fibrine and albumen, and that these materials are so completely changed within this organ that little or none of them leave it by the hepatic vessels, what becomes of them? It is true their hydro-carbonous constituents may be thrown out as bile; but what of the nitrogen contained in them? If it does not escape by the bile-ducts, it has no other mode of exit save by the hepatic vessels. The author conceives it to be re-united with the hydro-carbonous amyloid substance, and to leave the liver as a newly-formed proteic compound, partly, perhaps, as globuline, and partly as a material in its reactions resembling caseine or albuminose, and which shall be subsequently more fully described in this memoir. These considerations lead to the necessity of investigating the several distinct functions of the liver :—

1st. As to its action on the fibrine and albumen of the blood.

2nd. As to the constitution of healthy bile (so far as its azotised elements are concerned).

§ 3.

AS TO THE RELATION, COMPOSITION, AND CHARACTERS OF THE BLOOD WHICH ENTERS AND OF THAT WHICH LEAVES THE LIVER, AND SOME GENERAL CONSIDERATIONS AS TO THE VARIOUS FUNCTIONS PERFORMED BY THAT ORGAN.

(A) *Of the Functions of the Liver, as regards the Fibrine and Albumen of the Blood.*

It is probable that fibrine is *not* an agent essential to the nutrition of muscle—nay, more, there is a good deal of evidence to show that physiologists have erred in supposing that it is a formative material, necessary to the construction of muscular and other tissues. A considerable number of facts point in the very opposite direction, tending to prove that fibrine is, in some degree, the result of the disintegration of tissue. "I have proved," says Dr. Brown-Séquard, "that blood deprived of fibrine is as capable as normal blood of regenerating the vital properties or functions of various contractile or nervous organs. This is a fact which alone is enough to prove that fibrine is not an essential element in the nutrition of these organs, for it is to the nutrition, during the injection of the blood, that the return of the vital properties is due. If this fact is not admitted as sufficiently conclusive, I would mention an experiment undertaken to prove the following proposition—viz., that muscular irritability seems capable of being maintained for an indefinite period in limbs separated from the body, and into which blood charged with oxygen is injected. In one case, I have seen the irritability last for more than forty-one hours; in another, for more than fifty hours. But, if the irritability of muscle is due to its nutrition, which one cannot doubt, it is clear that nutrition may go forward in muscles supplied with blood deprived of fibrine, and consequently the fibrine is not, as has been generally believed, essential to the nutrition of muscular tissue."* Moreover, I have myself noticed, when repeating these experiments—at the time, not knowing that the same observation had been made by Dr. Brown-Séquard—that when defibrinated blood is injected into the arteries of a recently dead animal, it flows from the veins carrying with it some fibrine, so that it requires to be again defibrinated before it can be again injected. But, perhaps, the most important fact of all, in support of this view, is that pointed out by Lehmann†—viz., that it has been an error to suppose that in general arterial blood is richer in fibrine than venous; on the contrary, comparative experiments show that the blood in the smaller veins, and which has for the time done its work, contains a notably larger quantity of fibrine than that in the arteries.‡ A full consideration of the physiological characteristics of fibrine, tends more and more to establish the soundness of the view of its relations to tissue, long since put forth by Lehmann—viz., that it represents a transition stage, being in part related to the so-called progressive metamorphosis of tissue, and in part to the retrogressive. For, on the one hand, he has correctly maintained that this substance must be necessary to the formation of tissues, since, generally speaking, the only organisable exudations are those containing fibrine; while, on the other hand, we have before us the facts already stated, as well as the circumstances, that an increase of the

* Journal de la Physiologie, &c., tom. i. p. 731.

† Canstatt's Jahresbericht, 1855, B. 1, p. 183.

‡ Mr. John Simon, in his Lectures on Pathology, puts forth and ably advocates the view that fibrine is among those constituents which have arisen in the blood from its own decay, or have reverted to it from a waste of the tissues.

fibrine in the blood coincides with those inflammatory states, in which nutrition is most affected, and also that a similar increase takes place when albuminoid matters are taken in quantity above what is required for the repair of worn-out tissue. It is mainly, however, with that aspect of the question concerning fibrine which regards it as a product of the regressive metamorphosis of tissue, that we have to do at present; but in using the terms *progressive* and *regressive*, I would not be understood to adopt the view held by some, that certain elementary matters, entering the animal organism from without, of necessity mount up step by step, until they at last culminate in the formation of tissue, and having done so, then descend step by step, until eliminated from the system; that, for instance, the nitrogen entering the organism advances by a series of *progressive* metamorphoses, until each atom reaches its culminating point in azotised tissue, and subsequently, by a *regressive* process, leaves the body in the excreta as urea, &c., and that thus the amount of nitrogen excreted is an exact index of the amount of azotised tissue disintegrated. This view I conceive to be purely hypothetical, and, indeed, opposed to many well-known facts.

Whatever may be the origin of fibrine, it, at all events, in a great degree, disappears in the liver, or at least must undergo a very great modification within this organ.

Lehmann* is very distinct in his assertion, that the blood of the *portal vein* in horses and dogs contains fibrine which does not sensibly differ, in its characters and amount, from the fibrine of other veins; but that the blood of the *hepatic veins*, when carefully collected, contains no fibrine. He adds, that very careful comparative analysis of the blood of the portal and hepatic veins, has proved that a remarkable quantity of albumen also disappears in the liver.

The statement of Brown-Séquard is equally precise on the same subject. I have assured myself several times, he says, that the blood of the hepatic veins in dogs is not capable of coagulating spontaneously, and that it does not give fibrine on being whipped.† It may appear unnecessary to add my testi-

* Bernard Lecons de Physiologie Experimentale, cours du Semestre d'Hiver, 1854-55, p. 465.

† Journal de la Physiologie, &c., tom. i. p. 299.

mony in support of such evidence. I would merely add, that I have experienced great difficulty in obtaining blood from the hepatic veins unmixed with that of the cava, but that when care is taken to obtain it so, it appears to be devoid of fibrine. If, in a dog just killed, the abdomen be opened with as much speed as possible, and the hand introduced so as to grasp the mouths of the hepatic veins, if the liver be then removed, and the blood collected from these vessels by gently pressing the liver so as to squeeze it out, this blood will coagulate slightly; but if, when the animal is killed, a ligature be adroitly placed so as to tie up the mouths of the hepatic veins, and then, without any pressing or squeezing of the liver, the surrounding parts are so completely cleared away, that the blood which distends the hepatic veins can be drawn from them by a simple puncture, the blood so obtained contains no fibrine. This absence of fibrine is one of the circumstances which makes it very difficult to examine the matters contained in the serum of hepatic venous blood; for as this kind of blood does not coagulate, or coagulates very slightly, it is almost impossible to get from it serum which is not deeply stained with the colouring matter of the blood discs. For many purposes, therefore, it is desirable to obtain blood which, though chiefly hepatic, is so mixed with that of the ascending cava, as to render it coagulable to such a degree as to enable one readily to obtain tolerably colourless serum. In such serum the albumen is markedly less abundant than in the serum of the portal blood, although this latter contains, both absolutely and relatively, far more serum than hepatic venous blood. The duty which the liver thus performs, of more or less completely destroying or decomposing these nitrogenous substances, must, if the large quantity of them existing in the blood be taken into account, be considered one of the most important functions of this important organ. Brown-Séquard* calculates 2690 grammes of fibrine is daily lost to the blood in passing through the digestive organs and the liver. If we suppose that this is that portion of fibrine which results from the retrograde metamorphosis of tissue, and which is undergoing its final disintegration in the liver, it is easy to conceive how that even a slight interference with this fibrine-destroying

* Journal de Physiologie, tom. i. p. 304.

function in certain diseased states, would cause a rapid increase of the fibrine in the blood; moreover, we have an explanation of the source from which some of the ingredients of the bile may be derived, and a very probable explanation of the source from which the sulphur of the bile may come. But what of the nitrogen? Is it to be found in anything like proportionate quantity in the bile?

When it is recollected how conflicting are the statements of physiologists with regard to the amount of bile secreted, and of physiological chemists as to its chemical composition, the difficulty will be obvious of forming any very precise estimate as to the quantity of nitrogen thrown out by this secretion. It is, however, quite certain, that it is very inconsiderable in quantity. It is probably quite over the mark to suppose that the dry residue of fresh bile would yield so much as five per cent. of nitrogen; yet, assuming this, if we take a large animal secreting enough of bile in twenty-four hours to give two ounces of solid residue, the nitrogen of this would not amount to fifty grains. With a view to determine the amount of nitrogen separated from the blood by the liver, my friend and colleague, Dr. E. W. Davy, has been good enough to examine for me the fresh bile collected from the gall bladders of sheep. He finds that 100 parts by weight of such bile, evaporated to dryness, give seven parts by weight of dry solid matter; 100 parts by weight of this perfectly dry solid matter, contain only 3·90 parts of nitrogen: 100 parts by weight, therefore, of fluid bile, contain not more 0·273 parts of nitrogen.

The specific gravity of the bile which I obtained for the foregoing examination was 1023, which is a little below that usually given for this fluid; it consequently contained somewhat less soluble matter than usual. This, however, does not materially alter the general result, which comes to this, that a fluid ounce of sheep's bile, weighing 466·5 grains, contains no more than 1·273 gr. of nitrogen. However opinions, then, may differ as to the number of ounces of bile secreted in twenty-four hours, at all events the entire quantity of nitrogen thus separated from the blood is but small.

We are justified, therefore, in the assumption, that the nitrogen leaving the liver in biliary secretion, accounts for but a very small part of that which enters the liver by the hepatic artery and portal vein, and which must leave it by some channel.

If the view which I advocate with regard to the ultimate destination of the amyloid substance of the liver be correct, it is obvious that a direct relation must exist between the formation of this substance and the secretion of bile. If the liver, by disintegrating the fibrine of the portal blood, supplies, on the one hand, the materials of the bile, and on the other, the nitrogen for ultimate union with the amyloid substance, it is clear that those animals which provide most amyloid substance to unite with the nitrogen will also produce most bile; and we find that it is so. It has been already stated that the liver of—

The cat, gives, on an average,	1·5	per cent.	of amyloid substance.
And that of the rabbit	,,	3·7	,, ,,

Relying on the results obtained by Bidder and Schmidt, we find that those same animals secrete bile in a nearly corresponding ratio. For every pound weight in the body of each, they secrete during twenty-four hours—

The cat, of fresh bile,	102 grs.	giving 5·712 grs.	of solid residue.
The rabbit ,,	858 ,,	giving 17·290 grs.	,,

Again, admitting the diversity of opinion with regard to the period at which the secretion of bile goes on with most activity, we may, at least, arrive at the general conclusion, that, at the time when digestion is being vigorously performed, then not only is the bile very abundantly poured forth, but, at the same time, the amyloid substance is being abundantly formed.

One circumstance would, at first, seem to militate strongly against the view which I am inclined to adopt. It is this: we know that the liver forms the amyloid substance largely in animals fed on a purely saccharine and amylaceous diet; we also know that such a diet causes a great diminution in the secretion of bile. The true explanation of these apparently contradictory facts I take to be, that on an amylaceous diet less fibrine* is formed in the blood; the regressive metamorphosis

* Lehmann found that his own blood contained less fibrine when he was living on a vegetable diet than on animal diet, and Nasse has made experiments on dogs with a similar result.—Lehmann's Physiological Chemistry, vol. i. p. 358.

of tissue, resulting in an increase of the fibrine, is checked from want of nitrogenous food; hence, the fibrine-destroying function of the liver is also checked. As a result, less bile is secreted, and less nitrogen supplied for the azotisation of the amyloid substance, which, in consequence of not being used in the normal way, accumulates in the tissue of the liver. The fact that the urea excreted is remarkably diminished by an exclusively amylaceous diet, and that the urine of the carnivora* is so much richer in this material than that of the herbivora, points obviously in the same direction as the foregoing.†

Comparison between the Blood which enters and that which leaves the Liver.

It is a tolerably easy matter to obtain enough of portal blood for chemical examination. If the portal vein is expeditiously tied, in order to prevent regurgitation from the liver, and the vein, or some of its larger tributaries, opened below

* The quantity of urea in cats urine, as compared with rabbits, is enormously large. The specific gravity of the former I have found as high as 1070, while it gave 7.746 grains of urea in each fluid dram, or nearly 62 grains to the fluid ounce.

† A very interesting memoir relating to the cholesterine eliminated by the liver, has appeared in the American Journal of Medical Science, from the pen of Dr. Austin Flint, jun., Professor of Physiology to the Bellevue Hospital Medical College. It is entitled, "Experimental Researches on a New Excretory Function of the Liver, consisting in the Separation of Cholesterine from the Blood, and its Expulsion from the Economy under the form of Stercorine (Seroline of Boudet)."

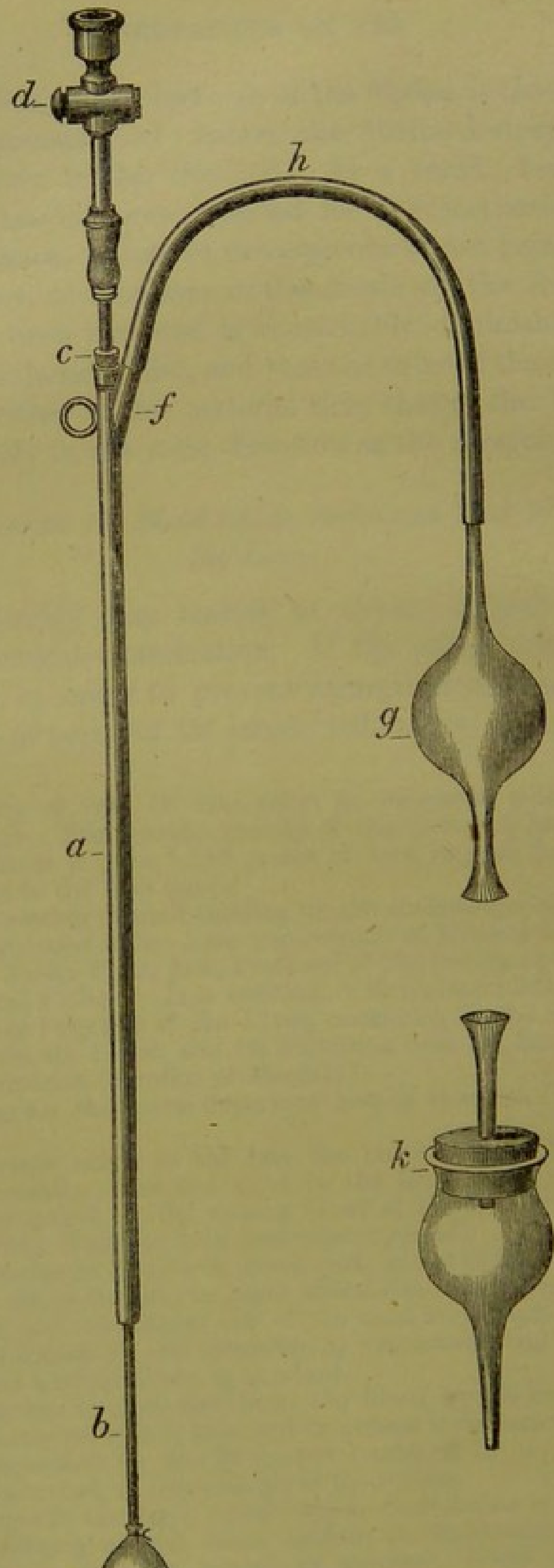
The following are the more important results at which Dr. Flint has arrived :—

1st. Cholesterine exists in the bile, the blood, nervous tissue, meconium; but, normally, does not exist in the fæces. The quantity of chlolesterine furnished by the venous blood of the arm, is from five to eight times greater than has been generally supposed.

2nd. Cholesterine is formed in great part, if not entirely, in the nervous tissue, in which it exists in great abundance. It is separated from it by the blood, and constitutes one of the most important excremential products of the economy. Its formation is continuous, and its existence in the blood and nervous tissue is constant.

3rd. Cholesterine is separated from the blood by the liver. It is an element constantly present in bile, and is poured forth into the digestive tube. It is separated by the liver, not produced by it; and, if this separation is disturbed, it accumulates in the system.

4th. Stercorine is the form under which cholesterine is ejected from the body. Ordinary normal fæces contain no cholesterine; but they contain stercorine (formerly known as seroline), which is a product resulting from the transformation of the cholesterine of the bile during the digestive process.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the ligature, a considerable quantity can be readily collected. There is, on the other hand, considerable difficulty in obtaining a good supply of the blood coming from the hepatic vessels. I have already observed, in the early part of this memoir, that when trying on dead animals the operation of introducing a catheter into the vein in the neck, for the purpose of abstracting blood from the right side of the heart, I sometimes found that the catheter passed by the heart, and went down the ascending cava. This, which first occurred by accident, I found could generally be accomplished at pleasure by a little manœuvring. This suggested to me a plan by which I have succeeded in obtaining specimens of blood, which, although by no means pure hepatic blood, yet is mainly derived from the mouths of the hepatic vessels. The accompanying drawing will make plain the form of instrument used for this purpose. It consists of a straight, or nearly straight (for it is occasionally necessary to bend it a little), silver canula (*a*), through which a small-sized gum-elastic catheter is passed. The gum elastic catheter fits very loosely into the silver canula, except at its upper end (*c*), where it is made to fit tightly. This is managed by a little piece of vulcanized indian-rubber tube, which makes the gum-elastic catheter fit the upper end of the silver canula, so as to be air-tight; but, at the same time, when the catheter is oiled, it slips freely up and down through this stopper of vulcanized indian-rubber. At one end of the gum-elastic catheter (*d*) is a small stop-cock, which opens and shuts with a spring, and at the other end (*e*) a small bladder, capable of being inflated by blowing in from the stop-cock. The gall bladder of a hedgehog, or some such small animal, suits well for this part of the instrument. At one side of the silver canula, is an offset tube (*f*), to which a pipette (*g*) can be attached, by the help of a piece of vulcanized indian-rubber tubing (*h*). If the instrument be dipt into water, so that the end of the silver canula be immersed, the water can be sucked up into the pipette, passing alongside of the gum-elastic catheter which is too small to fill the silver canula. When about to be used the little bladder is to be softened with water, and emptied of air. Then by drawing up the gum-elastic catheter through its air-tight stopper at (*c*), the bladder is drawn just inside of the silver canula.

A good-sized dog is to be selected for experiment: the head being bent backwards slightly across a cushion about as thick as the arm, the jugular vein is to be exposed for about an inch, and two ligatures passed beneath it. These ligatures are to be raised by an assistant so as to imprison some blood in the portion of the vein between them; thus distended, the vein can be opened to a sufficient extent to admit the end of the silver canula, while raising the ligatures prevents hæmorrhage. By a little management, the silver canula, thus introduced, is pushed downwards past the heart into the ascending cava. When it has arrived at the point (ascertained by measurement from dead animals) where the hepatic veins open into the cava, it is to be passed no further; but, now, the gum-elastic catheter is to be pushed out for one inch, by blowing through the stop-cock: the little bladder is now to be inflated, and so the vena cava is plugged a little way below the mouths of the hepatic vessels. Blood may now be sucked into the pipette through the silver canula. Two or three ounces may be thus obtained, for the most part coming from the liver, although mixed with that from above. On opening the stop-cock, the air escapes from the bladder, and the instrument may be withdrawn. It is convenient to have some pipettes made of the form of that drawn at (*k*) where the upper tube passes through a cork to facilitate the removal of the blood, which often coagulates before the experiment can be completed.

The blood obtained in this way, as has been already said, is by no means blood derived from the hepatic veins alone; it is, although mainly derived from the liver, sufficiently mixed with blood which has not been submitted to the action of this organ, to coagulate pretty firmly. A considerable quantity of clear serum can thus be got from it, and this serum is found to contain, more abundantly than other blood, a protein compound resembling what has been described by some as blood caseine;*

* MM. Dumas and Cahours have published in their memoir on Protein Compounds, the analysis of a product extracted from blood, possessing, if not all the properties of caseine, at least its composition. M. Stas has found a notable quantity of caseine in the blood of the placenta. Guillot and Leblanc have observed that the serum of several varieties of blood, when deprived of albumen, by boiling and filtration, furnished an abundant white precipitate when boiled with some drops of acetic acid. In this precipitate they recognised the characters of caseine.

a compound which, although in some respects resembling caseine, yet is not identical with it. In order to obtain this material, the serum is to be diluted with ten times its bulk of distilled water, and on then adding, cautiously, very dilute acetic acid (one part of the glacial acid to fifty of distilled water), so as slightly to acidulate the whole, a white precipitate is formed, which slowly subsides. That this material is an azotised compound is shown by the characteristic odour emitted when it is burned on platinum foil; that it is not simply albumen is proved by its being precipitated and re-dissolved by agents (acetic acid, ammonia, &c.), which do not thus affect albumen, and by the fact, that when it is abstracted from a known quantity of serum, this still yields as much albumen as an equal quantity from which this material has not been taken away. It is probably the same substance which has been noticed by various authors, under different names, as existing in the blood.* It is to be found in the serum of all blood; but what I desire particularly to point out is, that it is much more abundant in the blood found leaving the liver during the period of digestion than in any other specimens of blood I have examined. Next to the blood of the hepatic veins, I have found the blood of the placenta richer in it than ordinary venous or arterial blood. It may be that the material in question has been confounded by Lehmann with the cell walls of the blood corpuscles of the hepatic veins, when he writes: "But we may readily convince ourselves by the microscope of the almost entire absence of fibrine in the cruor of hepatic venous blood, and by the following experiment of the accuracy of this view, and of the great number of indestructible corpuscles in the blood of the hepatic veins. On mixing the fluid expressed from the clot with twenty times its quantity of water, *portal blood*, like that of any other vein, yields a slight flocculent deposit, in which shreds of conglomerated cell-walls may be

* *Natalis Guillot and F. Leblanc:* Note sur la presence de la caseine et les variations de ses proportions dans le sang des hommes and des animaux. Compts Rend. t. xxxi. p. 585.

Panum: Ueber einem konstanten, mit dem Caseïn überreinstimmendem, Bestandtheil des Blutes Archiv für pathol. Anatomie von Virchow, und Reinhardt, iii Band, 2 heft, p. 251; also, Band iv. heft i. p. 17.

Mialhe: Note sur la presence de l'albuminose dans le sang, &c. Compt. Rend, t. xxx. p. 745.

recognized by the microscope : if, on the other hand, we treat an equal volume of fluid strained from the cruor of *hepatic venous blood* with twenty times its quantity of water, there will be a flocculent precipitate of six or eight times the bulk of the precipitate in the other experiment."* I should add, that a dilution of the serum of blood with fifteen to twenty times its bulk of distilled water, is sufficient of itself to give rise to the precipitation of the protein compound already mentioned, without the addition of dilute acetic acid.

A large dog, six hours after having been fed on white bread and milk, and tripe, was submitted to the operation already described, for the abstraction of blood from the neighbourhood of the mouth of the hepatic veins. Three ounces (nearly) of blood was obtained from this source. Some was then drawn, first, from the jugular vein, second, from the carotid artery, and lastly from the vena portæ. Equal quantities of the clear serum, derived from each of these four samples of blood (200 minims of each), were each treated as follows : Each was diluted with ten times its bulk of distilled water ; very dilute acetic acid (1 minim of the glacial acid to 100 of distilled water) was added to each so long as any turbidity was produced. The precipitate thus formed in each was collected on a filter, dried and weighed. The following was the result :—

	Of the protein compound.
1st.—200 minims of the serum of the blood drawn from near the mouths of the hepatic veins, contained - - - - - - -	1·2 grs.
2nd.—200 minims of the serum of the blood taken from the jugular vein, contained - -	·4 grs.
3rd.—200 minims of the serum of the blood of the carotid artery, - - - - -	·8 grs.
4th.—200 mimims of the serum of the blood of the portal vein, - - - - - -	·4 grs.

It appears, therefore, that this material, which much resembles globuline, is most abundant in the blood coming from the liver

* Physiogical Chemistry, Cavendish Society, vol. ii. p. 107.

at the time of digestion, and least abundant in venous blood, while arterial blood contains it in larger quantities than venous. There is no marked difference in the quantity found in the blood of the porta, and the blood of other veins. I need hardly say, that the operation of abstracting blood from near the mouths of the hepatic veins, is one requiring great nicety, and that oftentimes it fails; but I have, on three distinct occasions, as I believe, perfectly succeeded, and on each have obtained results closely corresponding with the experiment detailed.

The protein compound already mentioned is either quite insoluble, or very sparingly so, in distilled water, in which it may be kept for several days without appearing to undergo any change; in this state it is not so prone to suffer decomposition as such compounds usually are. It is readily dissolved by dilute acetic, muriatic, and sulphuric acids, but not so by nitric acid. It is precipitated from its solution in dilute acetic acid by ferrocyanide of potassium. When well washed with distilled water, and collected on a paper filter, it is found, after exposure to the air, to undergo a very decided change—it loses its white flocculent appearance, and becomes transformed into a transparent, very viscid, gummy mass. In this state it is quite soluble in water, and, like the albuminose of Mialhe, is not precipitated by boiling, by dilute acetic, sulphuric, or muriatic acids, but is thrown down by corrosive sublimate or infusion of galls. It is entirely soluble in an alcoholic solution of potash, but is not soluble in alcohol. It is not identical with the material obtained by Guillot and Leblanc from the serum of blood, which is not soluble in dilute acetic acid. Although resembling caseine in its precipitation by dilute acetic, and its dissolving again in an excess of the same acid, I have not been able to cause its coagulation by rennet.*

Whatever may be its precise chemical composition and characteristics, whether it is to be regarded as a form of

* If the serum of blood be treated with a considerable bulk of alcohol, so as to precipitate all the albuminoid matters contained in it, and, if the precipitate, arrested on a filter, be boiled for some minutes with distilled water, a solution may be obtained which, when filtered off, is milky, and yields, on the addition of dilute acetic acid, a copious white precipitate. This is apparently identical with the blood caseine of Guillot and Leblanc.

albumen, or albumen-peptone (albuminose), or caseine, it is enough for me to state that, during active digestion, the blood which leaves the liver contains this compound; that it contains more of it than arterial blood; and that this latter is richer in it than ordinary venous blood, or than that of the portal vein. At the same time, the blood of the hepatic veins contains a far larger quantity of colourless blood corpuscles than the portal blood. A microscopic examination of these kinds of blood, soon convinces one that the colourless corpuscles are from five to ten times more numerous in the former than in the latter: physiologists are so familiar with this fact, as well as with the chief peculiarities of the colourless corpuscles of hepatic blood, that it is unnecessary to dwell upon the circumstances which have induced some of the most distinguished among them to regard, as the most important function of the liver, the formation, or at least the rejuve nescence, of the blood corpuscles. Dr. Carpenter conceives that the appearance of the colourless corpuscles of the blood may be regarded as a phenomenon analogous to the development of cells in the albumen of seeds in the vegetable kingdom. He also supposes that these cells aid in the conversion of crude alimentary matters into proximate principles. Additional support is given to each supposition by the notion, that these colourless cells stand in close relationship to the material formed in the liver so closely resembling dextrine of vegetable origin.

It is true that there is nothing novel in the view that the liver is a great blood-forming organ, or rather that it is an organ in which certain components of the blood are disintegrated, while from some of the matter so disintegrated a constant reconstruction of the blood is going forward; yet it is certain that, not long since, physiologists would have been unwilling to admit that materials constituted as the colourless blood-cells or caseine, could be formed within the liver from a substance resembling starch taking to itself nitrogen, derived, as one may say, from the retrogressive metamorphosis of tissue. It is very improbable that, looking to the liver alone, such a conclusion would have been arrived at. The consideration, however, of the physiological relations of the amyloid substance (of Bernard), as regards the development of the azotised tissues of the fœtus—the fact that it is, so far as they are concerned, a protoplasma, which, by taking to itself nitrogen, terminates in the evolution of fully-formed nitrogenous tissue—prepares one to consider the idea that the liver evolves its proteic compounds during adult life by a somewhat similar process. To M. Charles Rouget we unquestionably owe the observation of the fundamental facts which lead to the foregoing conclusions; yet the author hopes that the recapitulation of facts in this memoir, will be found worthy of the consideration of physiologists; for he conceives that, not only is the view of the subject which he has ventured to adopt in harmony with a great number of hitherto unexplained circumstances, but that it gives a solution more satisfactory than any yet given of a number of pathological phenomena.

THE END.

PRACTICAL OBSERVATIONS

ON THE

PATHOLOGY, PREVENTION, AND TREATMENT

OF

ASIATIC CHOLERA.