there are two essential processes in the coagulation of the blood, one of which has been hitherto entirely wrongly appreciated or overlooked. This latter process is that the "dead" plasma converts the white corpuscles directly into fibrin. At the same time, however, that this occurs, a substance is liberated from the cells which converts the fibrinogen also into fibrin. This is the other process. The substance which is liberated from the cells is fibrin ferment.

XVII. "A New Line of Research bearing on the Physiology of Sugar in the Animal System." By F. W. Pavy, M.D., F.R.S. Received June 8, 1881.

Twenty-three years ago I presented a communication to the Royal Society, entitled "On the alleged Sugar-forming Function of the Liver."

Four years previously, viz., in 1854, whilst conducting experiments directed towards determining the manner in which the sugar presumed, under the glycogenic doctrine, to escape from the liver was destroyed (as it was then believed to be) in the lungs, I discovered that what had been taken as representing the natural condition of the liver, and of the blood escaping from it in relation to sugar, was founded upon a fallacious inference. By those who have only been acquainted with what, in recent times, has been recognised as constituting the state existing, the original position in which the matter stood will hardly be fully comprehended. The strongly saccharine state in which the liver and the blood of the hepatic veins are found shortly after death was looked upon, without any question being raised about it, as representing the state existing during life. Without the slightest prior conception that such was likely to be the case, I found first that the blood between the liver and the lungs was not during life in the condition that had been supposed, and next that what I discovered for the blood applied also to the liver. The evidence which presented itself led me, as is known, to dispute the validity of the glycogenic theory, and the additional information which I have since from time to time obtained has materially strengthened the position I took. To my own mind, the conditions that we have to deal with looked at in their entirety, are totally irreconcilable with the glycogenic theory; but I know that the difficulty which has existed in accounting for the disposal of the glycogenic matter of Bernard encountered in the liver has stood in the way of a general adoption of my views. This subject, however, I am now prepared to approach and consider.

When the glycogenic matter was discovered, it was described as undergoing transformation into sugar immediately it was brought into

contact with blood. Bernard said of it "Cette dernière [matière glycogène du foie] est tellement alterable qu'elle ne peut pas exister dans le sang sans être immediatement changée en sucre, de sorte qu'elle ne peut jamais sortir du foie que sous cet état." This impression has governed the position left open to us to take in relation to the disposal of the material, and rendered it necessary to look for some undiscovered mode of transformation of it within the liver under the presumption that it does not reach the circulatory system as sugar.

We have here a fundamental point to deal with; and, as in my original communication to the Society, I had to commence by clearing the ground of error, so now it happens that I have to proceed in a similar way in relation to the point in question. I have results to communicate which appear to me to place us in a new position, but before these are considered it is requisite that the knowledge upon which we start should be set right.

Whatever may be the future of the glycogenic theory, the substance which Bernard was the first to recognise will stand as it exists now. The name "Glycogen" was applied to it when certainly an erroneous notion existed regarding the condition of the liver and of the blood in relation to sugar. Its presumed destination suggested the application of the term. I have hitherto objected to its employment, and I have stronger grounds for doing so now. To avoid the incorporation of theory, I have spoken, in my previous writings, of the body in question under the provisional name of "amyloid substance." Something more definite than this is for final purposes required, and it appears to me that it would be a fitting tribute to the memory of its discoverer to call it "Bernardin." Such a name will be at once suggestive of the substance to which it is intended to refer, and will form an imperishable memorial, which, whatever doctrine may prevail, will serve to identify the person, to whom all must admit physiological science owes so much, with the subject which formed the most prominent field of his labours.

Entering now upon the results which it is the object of this communication to make known, I will begin with the experiments bearing on the effect of bringing Bernardin (glycogen) into contact with blood.

In these experiments the Bernardin was dissolved in a small quantity of water, and mixed with the blood in a defibrinated state. Sheep's blood was the kind of blood used. The product was placed in an oven specially constructed for such a purpose, and maintained for half an hour at a temperature between 100° and 110° F. It was then subjected to examination, and the quantity of sugar present ascertained by the ammoniated cupric test.*

* The ammoniated cupric test referred to was described by me in former communications published in "Proc. Roy. Soc." vol. 28, p. 260, and vol. 29,

In some instances, the process of preparation for titration with the test consisted of extraction with alcohol, and in others of precipitation of the albuminous and colouring matters by heat with the aid of sulphate of soda. Bernardin (glycogen) prepared in different ways was used, and no difference in the results was perceived. Some of the specimens employed were obtained by simple extraction from the liver with water and precipitation with spirit. The actual condition of the specimen used as regards purity was ascertained by subjection to the converting action of sulphuric acid and heat, and the estimation of the glucose formed. In this way, the information was obtained for supplying the figures found in the subjoined table representing the Bernardin used, expressed in its equivalent of glucose. The figures representing the sugar produced were obtained by deducting from the sugar found in the product of experiment the figures yielded by a specimen of the blood alone purposely exposed to parallel conditions.

Bernardin (glycogen) added to Blood and exposed for half an hour to a temperature of 100° to 110° F.

	Amount of blood used.	Amount of sugar producible from the Bernardin mixed with the blood.	Sugar produced.
	cub. centims.	grm.	grm.
Experiment 1.....	60	0·267	0·007
„ 2.....	60	0·267	0·018
„ 3.....	30	0·535	0·005
„ 4.....	30	0·535	0·005
„ 5.....	25	0·232	0·009
„ 6.....	50	0·432	0·014
„ 7.....	50	0·288	0·021
„ 8.....	50	0·144	0·007
„ 9.....	50	0·294	0·009
„ 10.....	50	0·147	0·009
„ 11.....	50	0·144	0·036
„ 12.....	50	0·144	0·033
„ 13.....	25	0·144	0·007
„ 14.....	50	0·144	0·009
„ 15.....	25	0·144	0·000
„ 16.....	25	0·112	0·003
„ 17.....	25	0·112	0·002

p. 272. Since bringing it under the notice of the Society, I have had a very large experience with it, and am thus enabled to speak in definite and confident terms about it. Its facility of application, delicacy, and precision place us in a most advantageous position in relation to the quantitative determination of sugar. An important feature also belonging to it is that its action is not interfered with by the presence of nitrogenous matter as is the case with the ordinary cupric solution. Under my supervision it has been used, I am quite within bounds in saying, some

The total of these results is that Bernardin (glycogen) equivalent to 4·085 grms. of glucose was added to 705 cub. centims. of blood, and that the amount of sugar produced was 0·194 grm. I do not know the reason of the figures standing so much higher in experiments 11 and 12 than in any of the others. The two were conducted together and with the same specimen of blood, which was only used in these particular experiments.

The amount of sugar produced is too insignificant to be susceptible of recognition by ordinary testing. Half a gramme of a sample of Bernardin (glycogen), standing equivalent to 0·367 grm. of glucose, was mixed with 25 cub. centims. of blood, and exposed for half an hour to 100° F. Prepared for testing with heat and sodic sulphate in the ordinary way, the product gave with the cupro-potassic test no visible indication of the production of sugar.

The point shown by these experiments is that Bernardin (glycogen) may be brought into contact with blood and kept in contact, for some time, at a temperature equal to that of the living body, without undergoing conversion into sugar to more than what may be spoken of as quite an insignificant extent. Not only do the quantitative determinations of sugar prove this, but there is the further conclusive fact that the Bernardin is subsequently recoverable from the blood. The ground is therefore cleared in such a manner as to show that there is nothing inconsistent with Bernardin existing in and constituting a natural element of the blood.

Having established this foundation, I will now proceed to deal with the blood as it exists in the body, investigated in relation to Bernardin.

It is only when light begins to dawn upon a subject that the path of

thousands of times; and if an experience of this kind will justify an expression of opinion, I may state that I am satisfied it will be found invaluable alike to the physiologist, the chemist, and the physician.

The test is prepared by adding ammonia to a cupro-potassic solution, for which the following is the formula:—

Cupric sulphate	34·65 grms.
Potassic sodic tartrate (Rochelle salt)	170 „
Potash	170 „
Water	to 1 litre.

For the ammoniated test 120 cub. centims. of the above solution are mixed with 300 centims. of strong ammonia (sp. gr. 0·880), and water added to a litre. 20 cub. centims. of this ammoniated cupric liquid are decolorised by 0·010 grm. of glucose.

The liquid to be examined is allowed to drop from a burette into a suspended flask containing the test, which is kept briskly boiling till decoloration has been attained. The reduction being attended with decoloration without precipitation, there is nothing to obscure the determination of the moment when the precise point wanted has been reached.

investigation, which is calculated to lead to an extension of knowledge, appears in view. Until a definite purpose to work up to, suggested by something or other which has been extricated from the realm of darkness is attained, engagement in the laboratory operations of research is unsatisfactory employment. For some time, I persevered in conducting general analytical examinations of the blood and liver, hoping that, through the results obtained, light might appear in some direction or other, but they failed to lead to any useful acquirement of knowledge. Later on, from information supplied by a collateral course of inquiry, I was induced to conduct examinations by another method of procedure, which was not likely to have been hit upon accidentally; and through these a new field has been opened out, which, if I judge rightly, has given signs of proving productive of an important addition to our knowledge.

It would scarcely occur to a person, unless forced upon him by what he had otherwise observed, to think it necessary in relation to the matter under consideration, to preserve the coagulated residue of blood and submit it to examination. It will be seen, however, from what has to be stated, that besides a certain amount of glucose, which may be removed by alcohol, blood contains a principle which agrees with Bernardin (glycogen) in being insoluble in alcohol, and convertible into glucose by exposure to the influence of sulphuric acid and heat. Some of this principle is dissolved out by water under aqueous extraction, but the remainder clings to the coagulated residue, which has to be subjected to special treatment in order that it may be brought into view. Thus, unless a special mode of examination is adopted which is not likely to suggest itself accidentally to the mind, the principle in question incorporated with the coagulated residue will remain concealed from observation.

In conducting a full or detailed examination of blood, the first step to be undertaken is the separation of the glucose which it contains for quantitative examination. Let 25 to 50 cub. centims. of defibrinated blood be poured into five or six times their volume of spirit. The glucose being soluble in alcohol is susceptible of extraction by this liquid. It is held, however, more tenaciously by the coagulated matter than might be expected, and hence to effect a complete removal several washings and pressings are required. At first I was deceived through not fully realising this fact, and thought I had obtained evidence of the presence of glucose, of a maltose-like body giving a greater cupric oxide reducing action after being subjected to the converting influence of sulphuric acid and heat than before, and of a substance agreeing with Bernardin (glycogen) in blood. The first I found in the alcoholic extract, the second in the aqueous extract, and the third in the solid residue. The maltose-like body, I have since ascertained, has no real existence. The cupric oxide reducing

action in the aqueous extract noticeable before subjection to sulphuric acid and heat, arose from the glucose not having been thoroughly extracted in the alcoholic process, and the increased reducing action after sulphuric acid and heat was due to the presence, in addition to the glucose referred to, of a little Bernardin (glycogen) dissolved out from the residue by the water.

The plan that I adopt for effecting the complete removal of the glucose is as follows:—The defibrinated blood which has been taken for examination is poured into the requisite quantity of spirit, and the two are well stirred together. I am under the impression that it is advantageous for the coagulum to be allowed to remain in contact with the spirit till the following day. After being boiled by the heat of the water bath, the alcohol is strained off through a piece of linen material which has been cleansed so as to be free from dressing. Washing with alcohol is performed, and the coagulum in the linen is subjected to forcible squeezing in a suitable sized press. The residue, which by this process is converted into a dry cake, is pulverised in a mortar, mixed with fresh spirit, boiled over the water-bath, and again strained and pressed. The process is repeated once more, and this I find is sufficient. Thus extracted three times, there is practically no glucose left in the solid residue, and when an aqueous extract is made it gives no appreciable cupric oxide reducing action before subjection to the influence of sulphuric acid and heat.

Two extractions with alcohol might prove sufficient if carefully made, but it is safer to use three. To give the representations of actual results yielded, 50 cub. centims. of sheep's blood were extracted with alcohol. The first alcoholic extract contained 15 mgrms. of glucose, the second 4, and the third no definite amount. Some sugar from diabetic urine was added to sheep's blood, and 50 cub. centims. taken. The first alcoholic extract was found to contain 144 mgrms. of glucose, the second 9, and the third nothing definite.

To prepare the alcoholic extraction for testing, the mixture of alcoholic liquids obtained is acidified with acetic acid, heated to near boiling point over the water-bath, and then filtered through ordinary filtering paper. It is now brought down by heat to a small bulk, and treated with an excess of crystals of sulphate of soda with the view of causing the fatty matter finely dispersed through the liquid to agglomerate so as to be susceptible of removal by filtration. Water is added to the surplus crystals of sulphate of soda, and a hot solution made which is used for washing purposes.

The titration of the product of alcoholic extraction with the ammoniated cupric test gives quantitative results which stand in complete accord with those I obtained by the gravimetric process, which were mentioned in communications published in "Proc. Roy. Soc.," vol. 26, pp. 314 and 346.

For the purpose of ascertaining what kind of sugar is contained in the alcoholic extract, the product of extraction has been titrated with the ammoniated cupric test *before* and *after* subjection to the converting influence of sulphuric acid and heat. The resutts have shown that no increased reducing action is given after treatment with sulphuric acid and heat.

The cupric oxide reducing principle, therefore, which is extracted by alcohol from blood, consists of glucose.

When the alcoholic extraction has been thoroughly effected, the coagulated residue of blood fails, as I have stated, to yield to water anything possessing *per se* a cupric oxide reducing property. After treatment, however, with sulphuric acid and heat the aqueous extract is found to exert a certain amount of cupric oxide reducing action. The substance removed by water which possesses this property is part of the material present in the coagulated residue, which closely resembles, if it does not actually constitute, Bernardin (glycogen). There appears to be uncertainty in the amount extracted by water, and I think it may be considered that no useful information is derivable from the examination of the aqueous extract. Just for the purpose, however, of giving a representation of what I have found, I may state that taking the several observations of which I have a record (thirty-two in number), the amount of material contained in the aqueous extract convertible into cupric oxide reducing substance by sulphuric acid and heat, stands at about 0·290 per 1,000 expressed as glucose.

It did not occur to me, at starting, to do anything in the way of examining the residue left after alcoholic and aqueous extraction, not imagining that there would be anything of concern to me present. In the course of investigation, however, grounds presented themselves for leading me to deal with the residue, and I determined to treat it as I have been in the habit of treating the liver in making a quantitative determination of Bernardin (glycogen).

This process consists of dissolving by means of an alkali, pouring into spirit, and collecting the precipitate. Bernardin (glycogen) possesses two properties which greatly facilitate its separation from other bodies, viz., resistance to the action of an alkali and insolubility in alcohol. Albuminoid matters are attacked by caustic alkalies, and are then not precipitable by spirit as before. A method of separation is thus supplied. The object of applying the process was to ascertain whether there existed concealed in the residue anything of the nature of Bernardin (glycogen).

The residue was treated with water, and caustic potash added in the proportion of about one-fifth of the original weight of blood taken for examination. Heat was applied until solution occurred, and the product was poured into about five or six times its volume of spirit. The precipitate was allowed till the following day to settle, and the spirit

was then carefully decanted off and some fresh spirit added and time again given to settle. The next step was to purify the product, and this was done by collecting and dissolving it in a little water, thoroughly acidifying the solution with acetic acid, filtering from the precipitate produced, and then re-precipitating with spirit. The precipitate thus finally obtained was dissolved in water and examined. The solution was found to exert no reducing action upon the ammoniated cupric test before treatment with sulphuric acid and heat, but did so afterwards. Such is the behaviour of Bernardin (glycogen), and such behaviour, it is to be noted, was obtained from a product furnished by the process of preparation adapted for yielding in a separated form whatever of the principle in question the blood might contain.

I have spoken of the material I have been referring to, which is precipitable by spirit and convertible into glucose by sulphuric acid and heat, under the name of Bernardin, but I am not prepared to state that the principle which exists in the blood is absolutely identical with that belonging to the liver. The determination of this point must form the subject of future investigation.

The process I have described is the one by which I learnt that a principle agreeing with Bernardin (glycogen) may be recognised in the blood. A shorter method, however, may be had recourse to for revealing its presence. The blood may be at once treated with potash and the product poured into spirit. The subsequent steps to be followed are those which have been already described.

It is through the ammoniated cupric test that I have been led on to the acquirement of the information that I have obtained. The delicacy of the test, and the circumstance that its action is not interfered with by the presence of a small quantity of nitrogenous matter, have enabled me to discern conditions which the ordinary application of the cupro-potassic test would have failed to have revealed. Once in possession of the knowledge supplied through the ammoniated cupric test, I could see that the ordinary cupro-potassic test ought to be susceptible of being rendered applicable for revealing the glucose produced from the Bernardin (glycogen) existing in blood, and such, I find, proves to be the case. From half a litre to a litre of blood should be taken and treated by being at once boiled with potash and poured into spirit. The aqueous solution of the ultimate alcoholic precipitate obtained possesses, as will be understood, not the slightest cupric oxide reducing power of its own; but, after treatment with sulphuric acid and heat, and subsequent neutralisation, gives a good reaction with the cupro-potassic solution used in the ordinary way. The suitability of blood for operating upon to show this result varies, and a specimen should be procured from an animal in good condition and well fed up to the time of death. The blood obtained from the slaughter-house is

not always a favourable specimen for the purpose, and this is scarcely to be wondered at, looking at the abnormal state the animals often exist under for a day or two previous to being killed. Similarly, it is found that for collecting Bernardin (glycogen) from the liver, the slaughter-house often affords a very unfavourable specimen for the purpose.

I will furnish the results of the quantitative analyses I have performed. They were simply taken just as the specimens happened to present themselves. As yet I have not done anything towards ascertaining in a systematic way how the quantity of the principle is modified by antecedent conditions. This will form a subject for subsequent investigation. The glucose figures yielded by the analysis have been brought into Bernardin (glycogen) figures by the method of calculation which will be later on referred to.

Amount of Bernardin (glycogen) found per 1,000 of Blood.

Sheep.		Bullock.	Cat.	Rabbit.	Horse.
0·621	0·388	0·540	0·837	0·858	0·504
0·422	1·025	0·522	0·423		
0·540	0·605		0·387		
0·630	0·709		0·441		
0·549	0·594		0·630		
0·432	0·687		0·967		
0·623	1·483		0·693		
0·423	0·261				
0·797	0·283				

The mean of these twenty-nine results, taken altogether, gives 0·616 as the amount of Bernardin found per 1,000 of blood.

It has been seen that the method I adopt for the quantitative determination of the Bernardin present in the blood, is the conversion of it into glucose by the agency of sulphuric acid and heat, and then estimating the glucose by means of the ammoniated cupric test. This is the plan adopted for the quantitative determination of starch. I have experimented to ascertain the conditions required for securing complete conversion into glucose, and have found that Bernardin offers a far greater resistance to the converting action of sulphuric acid and heat than I had at first anticipated. The particulars of an observation are before me which showed that, after the process of boiling had been carried on for four hours, full conversion had taken place; whilst, at the end of three hours, conversion was considerably short of complete.

Fortunately for the progress of investigation, it happens that by exposure to a higher temperature under pressure, a comparatively short time suffices for effecting the complete conversion of Bernardin

into sugar. If the necessity existed for having recourse to such a prolonged operation as boiling for four hours, a serious obstacle would be offered to the performance of the quantitative determination to which we have to look for the supply of information. To shorten the time of exposure, I at first employed a stout glass flask with a vulcanised rubber bung wired into its mouth, and immersed this in an oil-bath heated to a considerably higher temperature than that of boiling water. Finding that I could not make sufficiently rapid progress with my investigations by proceeding in this way, I had a stout copper boiler constructed for the application of heat under pressure. It is provided with a lid which is screwed down with iron bolts, and has a lever safety-valve with a shifting weight to regulate the pressure. The boiler is large enough to receive a number of flasks, so that several products can be operated on at a time. I am in the habit of working it at 45 lbs. pressure, which gives a temperature just under 300° F. At this temperature, the conversion into glucose is accomplished within about a quarter of an hour. It is reliably secured by exposure for half an hour, and this time I am in the habit of allowing.

The quantity of sulphuric acid used is in the proportion of about 4 per cent. of the strong acid to the volume of liquid that is being worked upon. It is essential to give attention that the conditions are not such that the acid is appropriated to the liberation of another acid unendowed with the power of exerting a converting influence: such, for instance, as would occur if acetic acid had been previously used so as to leave a considerable quantity of acetates present.

From the amount of glucose, which, through the ammoniated cupric test has been ascertained to be produced, the quantity of Bernardin (glycogen) is calculated. The accepted formula for Bernardin is the same as for starch, viz., $C_6H_{10}O_5$. The formula for glucose is $C_6H_{12}O_6$. The equivalent of the one is 162 and of the other 180. From these we obtain the factor for the conversion of glucose figures into Bernardin figures:—

Let x = the amount of Bernardin equivalent to one part of glucose.

Then, as $180 : 162 :: 1 : x$,

or $$x = \frac{162}{180} = \frac{9}{10} = \cdot 9.$$

Hence ·9 stands as the factor to multiply by to convert glucose figures into Bernardin figures.

I have shown that after the extraction of glucose from blood by alcohol, there is to be found a material which gives no cupric oxide reducing action before treatment with sulphuric acid and heat but does so afterwards. Water dissolves out a certain portion of this

material; but the larger portion remains incorporated with the solid residue and is susceptible of being brought into view by boiling with potash and subsequent precipitation with spirit.

Bernardin (glycogen) added to blood behaves in a similar way. It is carried down, in great part, by the precipitated matter when coagulation is induced, and afterwards so tenaciously held that only the minor portion is removed by washing with water. The proportion dissolved out by water has varied considerably in different instances, and I have been led to question whether, in some cases, it may not be held to a more fixed extent than in others. I was at first much puzzled to know what had become of the substance, and it seemed, until I recognised it in the coagulated residue, that contact with blood led to an immediate extensive disappearance. To recover it, the coagulated matter requires to be subjected to disintegration by boiling with a caustic alkali. In some experiments, a notable amount of Bernardin has still remained unaccounted for, but, in some others, it has been nearly all recovered. For instance, in two recent experiments, after half an hour's exposure to a temperature of from 100° to 110° F., the following results were obtained: Bernardin equivalent to 0·112 grm. of glucose was in each case added, and Bernardin equivalent to 0·105 grm. of glucose in each case recovered. In another experiment Bernardin equivalent to 0·294 grm. of glucose was added, and Bernardin equivalent to 0·269 grm. of glucose recovered. I consider that further observations upon this subject require to be undertaken.

It is known that precipitates have a tendency to carry down other substances with them, and this principle, it may be suggested, accounts for what has been adverted to above. I am inclined to think, however, from a review of all the evidence before me, that there is something more than this at the foundation of what occurs. The question may be raised whether there is not some feeble combination existing, and whether this may not have a bearing of considerable physiological importance. Subjoined are results touching the point under consideration.

A weighed quantity of liver from a recently killed dog was reduced to a pulp and thoroughly extracted with alcohol for the removal of glucose. The coagulated residue from the alcoholic washing was then extracted with repeated washings of boiling water till the washings came away clear instead of lactescent as at first. The washings were collected and the Bernardin (glycogen) estimated by conversion into glucose and calculation from the glucose found. After being thus washed the liver residue was kept in a moist condition till the following day, when the process of washing and estimation of the extracted Bernardin was again performed. The process was subsequently repeated in a similar way; and, finally, the liver residue which had been

subjected to the several daily washings was boiled with potash and the liquefied product poured into spirit and the Bernardin collected and estimated. The following are the figures that were yielded :—

	Per 1,000 of liver.
Bernardin extracted the 1st day.....	13·833
,, ,, 2nd ,, ...	3·879
,, ,, 3rd ,, ...	2·961
,, ,, 4th ,, ...	2·817
Bernardin remaining in the liver residue ..	35·145

In another similarly conducted experiment upon the liver of a cat the following results were obtained :—

	Per 1,000 of liver.
Bernardin extracted the 1st day.....	3·951
,, ,, 2nd ,, ...	1·134
,, ,, 3rd ,, ...	Trace
Bernardin remaining in the liver residue ..	3·609

The above liver, it will be seen, was poor in Bernardin ; and this is often noticed in the cat, unless the animal has been fed with milk or bread in addition to, or in lieu of, meat.

In a third experiment upon the liver of a rabbit the following figures were furnished :—

	Per 1,000 of liver.
Bernardin extracted the 1st day.....	13·503
,, ,, 2nd ,, ...	3·376
,, ,, 4th ,, ...	3·688
,, ,, 8th ,, ...	2·466
,, ,, 12th ,, ...	2·299
Bernardin remaining in the liver residue ..	73·167

These results show how imperfectly ordinary extraction with water removes Bernardin from the liver substance, and account for the small quantities which, perhaps, may have been noticed to have been obtained when aqueous extraction has been had recourse to for collecting it.

Before the above quantitative experiments were performed, I noticed the following circumstance, which, indeed, led me to undertake them. Some bruised liver substance was washed in a beaker with boiling water, till the water failed to present the slightest appearance of lactescence. The last washing was poured off, and the liver substance simply left in a moist condition in the beaker, till the following day. On then boiling it up with water, a highly lactescent liquid (from the extraction of Bernardin) was again obtained. A few repetitions of the washing resulted, as on the previous day, in clear liquid being yielded, and the residue placed aside as before in a moist state, gave rise on

the following day to a recurrence of the phenomena. In some way or other, the Bernardin is held in the tissue, and with the lapse of time is set free for being taken up by water.

Although what I have described is noticeable when the liver substance is extracted with water at 212° F., yet a very different result is attainable by extraction under pressure, at a temperature of 300° F. At this temperature, it is found that all the Bernardin is speedily extracted. The copper boiler or digester, which I employ for the conversion of Bernardin into glucose by sulphuric acid, has been made use of for the extraction of liver experiments at 300° F., and half an hour, as the following statement of results obtained shows, has sufficed for the full accomplishment of extraction.

Two weighed portions of rabbit's liver, which had been bruised to a pulp in a mortar, were treated with spirit, and washed and pressed for the removal of glucose. The solid residue was then, in the one case, boiled with a solution of potash, and the Bernardin precipitated with spirit, and afterwards estimated in the usual way by conversion into glucose. In the other case, the liver residue, after alcoholic extraction, was treated with water and extracted at 300° F. The aqueous extract was then boiled with a little potash, and the Bernardin precipitated with spirit and estimated by conversion into glucose. The figures yielded stood thus :—

	Bernardin per 1,000 of liver.
After solution of liver substance in potash.....	91 ·314
After extraction at 300° F.	93 ·015

In a second similarly conducted experiment upon a rabbit's liver, the figures obtained were :—

	Bernardin per 1,000 of liver.
After a solution of liver substance in potash	64 ·242
After extraction at 300° F.	68 ·040

In a third experiment upon a cat's liver, the results were :—

	Bernardin per 1,000 of liver.
After solution of liver substance in potash	30 ·735
After extraction at 300° F.	31 ·742

Whatever the precise explanation, it follows from the results which have been set forth that the manner in which Bernardin (glycogen) is held, or the condition under which it exists in the liver, is such that at a temperature of 300° F., it is readily and completely susceptible of removal by water, whilst the resistance offered to removal is at the same time sufficient to permit only partial extraction to occur within

moderate limits of time at the ordinary boiling temperature of water.

When referring to the Bernardin (glycogen) discoverable in the blood, I stated that I was not prepared to assert that the principle existing in the blood is absolutely identical with that belonging to the liver. There is a point of difference which I have noticed in relation to the effect of extraction with water at 300° F.; but whether this is due to a difference in the properties of the principle itself, or whether to the manner in which it is held by other matter, I am not yet in a position to decide. The Bernardin of the liver, as I have shown, is readily extracted with water at 300° F., whilst according to the experiments I have conducted, that of the blood is not similarly susceptible of removal.

I have not yet said anything about the structures of the body generally in relation to Bernardin. I have subjected the spleen, pancreas, kidney, brain, and muscle to the same kind of examination as I have adopted in the case of the blood, and have found in all a notable amount of Bernardin.

Muscle has long been known to contain Bernardin (glycogen), but the quantity I obtain by the potash process is decidedly greater than what I believe is generally thought to be present. From cat's muscle, in one of my observations, the analysis yielded upwards of 5 per 1,000. Horse's muscle has been known to be specially characterised as containing Bernardin, and 9 per 1,000 in one instance is the quantity I have obtained.

In the observations I have yet made upon the spleen, pancreas, kidney, and brain, the largest amount of Bernardin has been found in the spleen, and in one instance the quantity indicated was a little under 4 per 1,000.

Bernardin therefore has been found as a constituent of all the tissues I have up to the present examined. I have likewise obtained it in notable amount from both the white and yolk of egg.

Besides Bernardin, there is, in the several tissues I have referred to, a cupric oxide reducing substance, which is susceptible of extraction by alcohol, and this also is present to a somewhat notable extent. In the case of the spleen, pancreas, kidney, and brain, this body appears to be glucose, the reducing power in most of the instances having come out about the same before and after the treatment with sulphuric acid and heat. In the case of muscle, however, the reducing power has been usually observed to be about twice as great after the treatment with sulphuric acid and heat as before. It seems, therefore, that we have here a body of the nature of maltose, instead of glucose, to deal with.

Although I have a considerable number of observations before me, yet I consider it advisable, at present, only to speak in the general way

that I have done upon this last subject. Observations conducted under varied physiological conditions require to be undertaken, so that besides having the facts as mere chemical facts before us, we may be in a position also to deal with them from a physiological point of view. In a subsequent communication, I will enter further into this matter, and then supply details of the actual quantitative results.

Summary of Conclusions.

Bernardin (glycogen) does not undergo any significant transformation into sugar in contact with blood.

Bernardin exists to a distinctly notable extent as a normal constituent of blood.

The evidence derivable from the observations recorded on the addition of Bernardin to blood and its subsequent recovery, and on its extraction from the liver by boiling water on successive days, and by water at 300° F., tends to show that Bernardin enters into feeble combination with nitrogenous matter.

Bernardin exists in notable amount, not only in muscle, as has been previously known, but also in the spleen, pancreas, kidney, and brain. These are all the structures I have yet examined. It also exists in notable amount in the white and yolk of egg. These several products likewise contain a cupric oxide reducing substance, which is extracted by alcohol, and which, in most instances, possesses the characters of glucose, but, specially in the case of muscle, the characters of maltose.

Through the existence of Bernardin (glycogen) throughout the system, as has been represented, we have a carbohydrate occupying a parallel position to albumen, viz., existing in the colloidal state, and thus adapted for retention within the body, instead of passing off as a diffusible substance as glucose tends to do.

XVIII. "On the Stresses caused in the Interior of the Earth by the Weight of Continents and Mountains." By G. H. Darwin, F.R.S. Received June 11, 1881.

(Abstract.)

In this paper I have considered the subject of the solidity and strength of the materials of which the earth is formed from a point of view from which it does not seem to have been hitherto discussed.

The first part of the paper is entirely devoted to a mathematical investigation, based upon Sir William Thomson's well-known paper on the rigidity of the earth.* The second part consists of a summary and discussion of the preceding work.

* "Thomson and Tait's Nat. Phil.," § 834, or "Phil. Trans.," 1863, p. 573.