XXVI. *Researches on Sugar Formation in the Liver.* *By* Frederick William Pavy, *M.D.* *Communicated by Dr. Sharpey, Sec. R.S.*

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The following communication is an abridgement of a paper on the same subject presented to the Royal Society in 1858, with some additional matter that has been since disclosed by my experimental investigations. The original paper, being deposited in the Archives of the Society, is accessible for reference on points of detail that are here excluded.

In 1848 it was announced by Bernard that the liver enjoyed a sugar-forming function. This statement appeared to rest upon irrefutable grounds, and the new function soon became almost universally acknowledged by physiologists. An animal which had been for some time previously restricted to an animal diet was suddenly killed. Sugar was found abundantly in the blood of the *vena cava* and hepatic veins, whilst none existed in that of the portal vein. The tissue of the liver was also found abundantly saccharine, whilst no sugar was to be detected in any other organ. I had seen this experiment several times performed in Bernard's laboratory, and had often repeated it myself. From the correctness of the description of his results, I entertained no doubt as to the accuracy of Bernard's deductions, and did not for a moment seek to question them. In the course of my experimental research, however, I was conducted step by step to a point which has placed me, involuntarily as it were, in antagonism with the glycogenic theory. By pushing investigation further than had hitherto been done, I have been compulsorily brought to arrive at conclusions of which I had not the most remote anticipation beforehand.

More recently the existence of a material, allied in its nature to starch, has been discovered in the liver which has been looked upon as destined for transformation into sugar. This "amyloid substance" has been hence called by Bernard the glycogenic matter. The discovery of this substance did not alter his pre-existing views, but simply provided a recognizable source for animal sugar.

The question arising out of my experimental results to be mentioned, is not whether sugar can be formed in the animal system independently of a saccharine alimentation, but whether the sugar so largely met with in the liver and a certain portion of the blood after death by the hitherto adopted processes of examination is to be taken as representing an *ante-mortem* or physiological condition; and whether the liver enjoys the special sugar-forming function that has been assigned to it.

It was upon finding that blood withdrawn from the right side of the heart during life differed so much in the amount of sugar it contained from what had been inferred

from examination on removal after death, that I was led to inquire into the substantiality of the glycogenic theory. From the experiments I had seen performed in Paris, and those I had made myself, I had regarded the blood of the right side of the heart as naturally strongly charged with sugar. When collected without the observance of certain precautions, from the hepatic veins, inferior cava, right auricle, or right ventricle of the recently killed animal, the blood, after the usual preparation for analysis with the sulphate of soda, gives a copious yellow or orange-yellow reduction on being tested with the copper solution, and this was looked upon as representing the state belonging to life. In February 1854 I was led in some experiments I was then conducting, to draw blood by a catheter from the right ventricle of the living animal, and was astonished to find that the blood removed did not present what I had hitherto looked upon as the natural reaction of right ventricular blood, for it was scarcely at all impregnated with saccharine matter. In June of the same year, according to my note-book, I again removed blood from the right ventricle during life, and observed it to contain scarcely a trace of sugar; whilst, on the animal being immediately afterwards killed, the blood which flowed from an incision into the right heart gave the usual reduction with the Barreswil solution.

Notwithstanding that such results appear now so striking, yet I did not at first give to them their proper significance. My mind was so strongly impressed with the prevailing conviction that sugar was extensively formed by the liver during life and poured into the circulation through the hepatic veins, that I felt inclined to think the catheter had not been fairly introduced into the heart, or had come in contact with the current of blood descending through the superior cava, rather than discredit our previous notions, and no longer regard the strongly saccharine state of the blood met with so soon after death, as a representation of its natural or *ante-mortem* condition.

This question afterwards presenting itself strongly before me, I was anxious definitely to decide if death really occasioned an alteration, as to the presence of sugar, in the blood escaping from the liver. Such an effect, as far as I am aware, had never been spoken of, or even hinted at by others. Several experiments were performed with the greatest care. Blood was withdrawn from the heart during life by the introduction of a catheter, and immediately afterwards the life of the animal was destroyed and blood collected by a free incision into the auricle or ventricle. In two cases the catheter had perforated the heart, and the blood that was removed during life had escaped into the pericardium, thus placing beyond all doubt, that a fair sample had been obtained. In every experiment (and the experiments were most numerous) the result of the examination of the blood was the same. The blood yielded by the right heart during life, gave only the merest trace of saccharine reaction, no more than was met in the arterial system; whilst that which was collected after death reduced abundantly the copper solution.

In some experiments a quantitative determination of the sugar existing in the blood collected from the right side of the heart after death was made, and the following are the results that were obtained.

Blood from the carotid artery and from the right ventricle during life.	Blood (defibrinated) from the right side of the heart after death.	Liver a short time after death.
No. 1. Trace of sugar.	$\frac{7}{10}$ths gr. of sugar per cent.	Not analysed.
No. 2. Trace of sugar.	$\frac{65}{100}$ths gr. of sugar per cent.	4·10 grs. of sugar per cent.
No. 3. Trace of sugar.	$\frac{5}{10}$ths gr. of sugar per cent.	3·39 grs. of sugar per cent.
No. 4. Trace of sugar.	$\frac{94}{100}$ths gr. of sugar per cent.	2·45 grs. of sugar per cent.
No. 5. Trace of sugar.	$\frac{7}{10}$ths gr. of sugar per cent.	2·44 grs. of sugar per cent.

At the time the above analyses were made, I looked upon the amount of sugar in the right ventricular blood removed during life as too small for exact determination. I have since, however, found that although minute, yet, the quantity is with care susceptible of being ascertained. Defibrinated blood is poured into spirit, and the precipitate thoroughly washed with that agent. The filtered liquid is evaporated to a small bulk, and the sugar then estimated with the Barreswil solution. The following figures may be given as affording an average representation of the per-centage of sugar normally existing in living right-ventricular blood of the dog, for the specimens taken behaved, as regards degree of reaction, like those belonging to the extensive number of examinations that I have made.

Blood collected six hours after a meal of animal food; 710·1 grs. of defibrinated blood taken for analysis: sugar $\frac{47}{1000}$ths of a grain per cent.

Blood collected twenty-four hours after the last food had been given: sugar $\frac{73}{1000}$ths of a grain per cent.

Blood collected $4\frac{1}{2}$ hours after a copious meal: sugar $\frac{58}{1000}$ths of a grain per cent.

It is therefore evident that blood collected from the right heart, as has hitherto been done after death, affords an indication differing most essentially from that yielded by blood withdrawn from the same part during life. Consequently inferences of the *ante-mortem* state that have been drawn from *post-mortem* examinations must be abandoned.

With the knowledge we now possess, it is easy to operate and find the blood after death presenting the condition that is natural to life. When an animal is killed, and even only a short time is allowed to elapse before the chest is opened to collect the blood from the right heart, it is found strongly saccharine. But if life be instantaneously destroyed, and the chest as rapidly as possible opened, and the heart ligatured at its base and excised, then the contents of its right cavities will be found as free from sugar as if catheterism had been performed during life. Here the steps of the operation are so rapid that time is not given for the *post-mortem* effects on the liver and the circulating fluid to be manifested.

I think, perhaps, that this is even a preferable mode of operating; and if expeditiously performed, gives blood more free from sugar than after removal with the catheter during life. Very slight causes are sufficient to determine an increase of sugar in the circulation. Struggling on the part of the animal leads to compression of the liver by the abdominal parietes, and more or less congestion of the circulation, by interfering

with the freedom of the breathing. In resorting to catheterism of the heart, my experience is, that unless the animal has remained perfectly tranquil during the whole of the operation, the blood will give a decidedly stronger shade of saccharine indication than is obtained under a natural or tranquil state of the system.

According to the state of tranquillity or restlessness of the animal, I can predicate with confidence the condition its blood will present; and when the operation is unattended with the least attempt at resistance, which only happens occasionally, the amount of reaction is so small that it is liable to be overlooked altogether, unless special attention is given. It is for this reason that I can reckon with greater certainty on meeting with the blood of the right side of the heart in a perfectly normal state on suddenly killing the animal and instantly excising the heart, than on removal through the catheter during life.

This affords an explanation, why blood removed from the carotid artery immediately after exposing the vessel, is ordinarily found in a marked degree more saccharine than when collected ten minutes or so after the operation of exposing the artery has been effected. From the contiguity of the carotid artery to the pneumogastric nerve, picking up the vessel is almost universally attended with considerable struggling and disturbance of the breathing. But when the artery has been once fully separated from its adjacent structures and a ligature placed around it, it can be drawn out and blood collected without causing any fresh struggling or disturbance.

I have upon a few occasions found blood removed from the carotid artery giving a shade stronger reaction than the blood previously withdrawn from the right side of the heart. I consider this to have been due to the different effects of the respective operations required. Exposing the jugular and passing a catheter into the heart, is not so likely to occasion struggling and disturbance as exposing the carotid.

From some experiments I have recently performed, it would seem that the portal blood, instead of being entirely free from sugar, contains, as far as is discoverable by the behaviour of the blue liquid, the same amount that is met with under natural circumstances in the heart and other parts of the circulatory system. Blood is examined (certainly by myself) much more closely now, than when such a wide difference was considered to exist in it from different parts of the circulation. A trace of reaction is now looked for and noted that would have formerly passed over unobserved. For instance, on well-boiling a specimen derived from portal blood with the blue liquid, there is no reduction to be perceived. The test appears to have given no reaction, and such would have been formerly described as the result. Had the test-tube been placed aside, however, and examined again in the course of half an hour, just a traceable amount of red oxide would have been found, according to my recent experience, to have subsided. The following is the account of three consecutive experiments that I have lately performed, in which a careful comparative examination of the portal and cardiac blood was made. The dogs had been feeding on tripe for some days past. They were suddenly killed by pithing. The abdomen being instantly opened, a ligature was placed (observing the precautions mentioned by BERNARD) around the portal vein. The chest

was immediately afterwards laid open and the heart instantly excised, and the blood collected from its cavities. Both portal and cardiac specimens of blood behaved precisely as I have described above, namely, gave no perceptible reduction on boiling with the blue liquid, but occasioned a traceable amount of red oxide to collect on reposing for a short time. There was no recognizable difference in the amount of deposit produced by the respective specimens of portal and cardiac blood.

That obstruction to the breathing occasions an unnaturally increased amount of sugar in the circulation is not only proved by examination of the blood; but the amount may be so large as to lead to the production of a diabetic state of the urine. Thus, in a healthy dog at a period of digestion I have obtained the following result. The urine was first drawn off and tested, and gave no trace of reaction. The head was then muffled in a bladder for an hour, during which time the supply of air allowed was exceedingly limited. A state was induced just short of coma or unconsciousness, and when the bladder was removed the animal immediately revived. The urine after this operation gave an orange-yellow reduction with the copper solution.

Although the conclusions advanced in this communication stand in antagonism to the glycogenic theory of BERNARD, yet I consider there is only one essential point where our *experimental results* are at variance. It is not that the statements here brought forward contradict the accuracy of BERNARD'S experiments. There is no doubt about the correctness of these experiments, and my own views are perfectly compatible with them. It is the inferences drawn from them that I contend are fallacious. A *post-mortem* condition has been taken as representing an *ante-mortem* or physiological state. At first there was no reason to suppose that this would occasion any error, but by the investigations I am detailing, it appears that the two are distinct from each other. And when BERNARD speaks of blood removed by the catheter from the right heart of the living animal precipitating "très nettement le sel de cuivre*," here my experience is directly opposed to him. If a most extensive number of observations enables me to speak with decision, I can confidently assert that such is not the normal character of blood contained in the heart during life.

At the time of discovering the fallacy of inferring the physiological state of the blood from the character it presented when collected from the heart after death, I still regarded the liver as strongly charged with sugar during life. I did not as yet think that the saccharine state of this organ displayed by our hitherto adopted mode of examining it was due likewise to a *post-mortem* occurrence. My first step was by injections of blood through the liver at different pressures, so as to imitate different states of the circulation, to endeavour to account for the sugar escaping so largely immediately after death whilst it scarcely appeared during life. Failing to discover anything decisive from these experiments, I began to direct my attention to the state of the liver tissue itself during life. I did not regard it as probable, but still, just possible, that the liver, like the blood coming from it, might be free, or almost entirely free, from sugar during life, and that

* Leçons de Physiologie, Paris, 1855, p. 121.

the presence of this principle might be due to some *post-mortem* change. Acting upon this idea, I resolved to see if I could not alight upon the organ in the state actually belonging to life, by placing it in a condition at the instant of death that would prevent any subsequent production of sugar.

The researches of BERNARD having disclosed the existence of a material in the liver which passes with great rapidity into sugar under the influence of ferments, I looked for an agent that might possess the power of preventing this transformation, without destroying the material or its product. Saliva is an exceedingly energetic ferment towards this substance, but I found that it ceased to exert any catalytic effect in the presence of an alkali. I therefore injected a strong solution of potash into the portal vein instantaneously after the destruction of life, with the view of preserving the liver in its natural state. After this operation, I have failed to detect any, or more than a mere trace of sugar in the substance of the organ; and it has been proved, by afterwards submitting a liver in which saccharine matter has existed, to a precisely similar process, that the injection has no effect towards leading to a decomposition of this principle. A saccharine liver injected with potash gives as copious a reaction as if the injection had not been practised.

Acids, like alkalies, check the action of ferments on this substance belonging to the liver, and a strong solution of citric acid injected into the portal vein has been observed to be followed by a similar effect to the injection of potash.

Although I believe that these experiments may be relied on as affording accurate information regarding the physiological state of the liver, yet a feeling of hesitation might possibly arise in accepting the results derived from the employment of a caustic alkali, when the subsequent recognition of an organic substance like sugar is concerned. There are other means, however, that enable us to display the condition of the liver belonging to life. The mere alteration of temperature is sufficient to effect the object that is desired. Processes of the nature of fermentation are checked by cold; and as the formation of sugar in the liver is of this character, the rapid abstraction of heat instantaneously after the destruction of life prevents any *post-mortem* change taking place, and presents us with the organ in a natural or physiological state.

The experiment showing the effect of reduction of temperature is thus performed. An animal is killed as quickly as possible by pithing the *medulla oblongata*. The abdomen is instantly opened by a free incision through its parietes, and a piece of the liver sliced off and plunged into a freezing mixture of ice and salt, which has stood sufficiently long to have become liquefied. If the ice and salt are used immediately after being mixed, and whilst still in a solid state, their cooling effects are not nearly so intense, and not sufficiently so for our purpose. The piece of liver in the course of a few minutes is frozen quite hard. It is taken out, cut up into thin slices, pounded to a pulp in a mortar, and placed, a little at a time, in a rather small quantity of water, which is kept thoroughly boiling during the process. The cold has merely checked the action of the ferment existing in the liver. The materials are there in an undestroyed state,

and a production of sugar takes place on the temperature being raised to a moderate extent. The object, therefore, is to apply the heat rapidly, so as to coagulate the albuminous principles and thus destroy the ferment. The decoction that is obtained gives none, or at the most the merest trace of reaction. It abounds in the sugar-forming substance, and on being treated with a little saliva and exposed to a moderate heat, a transformation is with an astonishing rapidity effected. The remainder of the liver that has been left in the animal is now submitted to the ordinary process of preparation, and tested, to show that it gives the usual reaction that was formerly thought to belong to it during life.

The rabbit is a more favourable animal for this experiment than the dog, because, on account of its size and the thinness of its abdominal parietes, the liver can be more quickly reached and a piece of it sliced off. Again, the liver of the rabbit is not so thick as that of the dog, and thus is more rapidly acted on throughout by the freezing mixture. I have often, in the case of the dog, found the moderately thick outside parings of a piece of frozen liver free, or almost completely free, from sugar, whilst the centre has given a tolerable saccharine reaction. The centre of a thick piece of liver may be found, even after immersion for some minutes, in a soft or unfrozen state; and when the abstraction of heat is only gradual, time is given for the production of a certain amount of sugar.

Whether the animal, either rabbit or dog, is at a period of digestion or not, makes no difference, that I have perceived, in the result. Usually my experiment has been made a few hours after food has been taken. A piece of healthy frozen liver that is free from sugar, on being raised for a few minutes to a temperature of 90° or 100°, gives a most copious saccharine reaction. A piece of saccharine liver plunged into a freezing mixture loses none of its saccharine qualities.

The result produced by boiling water on the liver is similar to that of a freezing mixture. It coagulates or destroys the materials capable of acting as ferments, and so prevents the ordinary *post-mortem* transformation from occurring. Its action, perhaps, is not quite so perfect as that of freezing, but still, especially with the liver of the rabbit, specimens are easily obtained yielding only the faintest indication of sugar. On testing the decoction, there is no change on boiling with the blue liquid, but after some time a few particles of red precipitate may subside. The animal is suddenly killed, and a piece of the liver as rapidly as is possible removed and thrown into a large quantity of boiling water. It need not remain more than two or three minutes. Pounded in a mortar with water and boiled, a liquid is furnished on filtration for testing.

It has been noticed by BERNARD, that after division of the spinal cord just below the phrenic nerves, the liver is found free from sugar upon being ordinarily examined at the period of death, whilst it becomes strongly saccharine afterwards. Various suggestions have been made in explanation of this phenomenon. The fact itself is a most striking one, and has frequently been corroborated in my laboratory. It fully agrees with the experiments I have been mentioning, and receives from them, I consider, a satisfactory

explanation. After the division of the spinal cord, the temperature of the animal rapidly falls. In one case I noticed in a rabbit $3\frac{1}{2}$ hours after the operation, that the temperature of the rectum was only 67°. This low degree of temperature at the time of death renders the *post-mortem* production of sugar so slow a process, that it is easily recognized in its true light.

Maintaining artificially the heat of the animal by exposure to a high temperature after division of the spinal cord, altogether alters the result. Thus, a rabbit in which the spinal cord had been divided just below the phrenics, was placed in an atmosphere where the thermometer stood at 88°. In three hours' time, when it was killed by pithing, the temperature of its rectum was 104°. The liver behaved precisely as if the animal had been suddenly killed in its ordinary state, sugar being found, after an ordinarily conducted examination, to its usual extent.

It has also not escaped the observation of BERNARD, in experimenting on the frog, that, according to the temperature of the animal at the time of death, the liver is found with or without sugar after an ordinarily conducted examination. In the 'Comptes Rendus' of the Academy of Sciences, March 1857, he mentions this fact, and refers it to a variation in the activity of the circulation, produced by the high and low temperature leading to an increase or decrease, or even arrest of the glycogenic function. I had obtained results corresponding with those of BERNARD before I was aware he had undertaken his experiments. Frogs in good condition, in which the liver is large, pale-coloured, and exceedingly rich in amyloid substance, were exposed for two hours to an atmosphere heated to 90°. An examination of the livers in the usual way afforded a decided indication of the presence of sugar. In other frogs, without exposure to the elevated temperature, the livers similarly examined gave no reaction with the Barreswil solution. Whilst repeating these experiments, I accidentally met with a result for which I was formerly at a loss to account. Some frogs that had been exposed to heat happened to be placed aside in my laboratory for a quarter of an hour before they were killed. Their livers, being examined, yielded scarcely an indication of a trace of sugar. Time had been given for the temperature to fall; and from what has preceded, it will now be seen that the absence or presence of sugar in these experiments depends upon the influence that a high or low temperature has been shown to exercise over the *post-mortem* change that occurs in the liver.

I have found the livers of the oyster and mussel, when these animals are in a fresh and healthy state, to be free from the presence of sugar. But the livers contain a large quantity of the amyloid substance, and should the animals have been allowed to die, or have been kept for some time, sugar may be encountered to a large extent. The same is the result when the livers are removed and moderately heated a short time before the examination is made.

Immediately underneath the shell of the mussel (*Mytilus edulis*) is a layer—the mantle—which is highly charged with the same amyloid substance that is met with in the liver. When the animal is in good condition, this layer is thick and opakely white

or yellowish; but when in the opposite state, it is thin, transparent, and watery. This mantle, of good-conditioned mussels, yields a decoction, which is quite milky from the amount of amyloid substance that is present. There is no sugar, and scarcely a proneness in the mantle itself to produce sugar; but if a ferment, such as a little saliva, be added, in a few moments an abundant production of sugar is the result.

The amyloid substance appears, from a micro-chemical examination of the liver, to be located in the hepatic cells. It belongs only to the healthy or physiological state, and may therefore be regarded as the result of a specific functional formation. It is most prone to descend by a chemical process into sugar; but an examination of the liver after different diets shows that it is produced, certainly in part, during life from sugar and its ally, starch.

In making a quantitative determination of this material in the liver, I have availed myself of its properties of resisting the action of a boiling solution of potash, and of being precipitated by spirit. The following results were obtained by pounding a piece of weighed liver with potash, adding a little water, and boiling some minutes until all was completely dissolved. The liquid was then poured into about six times its volume of spirit, by which the amyloid substance was thrown down as a white flocculent precipitate. The precipitate, after being well washed with spirit, was dried and weighed.

The size of the liver is to a most striking extent influenced by the nature of the diet; and the alteration that is thus induced is chiefly, if not entirely, due to the amount of amyloid substance present. I was first conducted to the discovery of these facts, whilst in my early experiments I was seeking to determine if the quantity of sugar found in the liver and blood of the dog after death was altered from its ordinary amount by the previous administration of a strictly vegetable diet. The process I then adopted for examining the liver was to remove and weigh it, and then to pass a stream of water through its vessels until its tissue was completely deprived of sugar. After a vegetable diet, I noticed, first, that the liver was of enormous size, in comparison with what I had been accustomed to meet with under an animal diet; and secondly, that there was a remarkable quantity of a material present which much interfered with my analysis, and which I subsequently found to be amyloid substance.

In all the observations that follow, the life of the animal was suddenly destroyed, its body then opened, and the liver removed. The liver thus circumstanced immediately drained itself, by the contraction of its vessels, of the principal portion of its blood, whereby was avoided those differences, as regards degree of congestion, which are observable in the human subject, where the examination is not made until some hours after death.

The observations are given without a single exception, just as they presented themselves under their separate heads; and further, the animals were taken, as they happened to be brought to me, without any selection, except such as was needed for the vegetable and saccharine diet, many dogs refusing to partake of this kind of food. The weights are of the avoirdupois scale. The dogs were weighed just before death, and

the weight of the liver is without the contents of the gall-bladder. Life was in most of the instances destroyed a few hours after the administration of food.

The Liver of the Dog under a Diet of Animal Food.

	Weight of dog.		Absolute weight of liver.	Relative weight of liver to animal.
	lbs.	oz.	oz.	
No. 1 . . .	15	8	$7\frac{3}{4}$	1 to 32
No. 2 . . .	12	0	$7\frac{1}{4}$	1 to $26\frac{1}{2}$
No. 3 . . .	11	$14\frac{1}{2}$	$6\frac{1}{8}$	1 to 31
No. 4 . . .	15	10	$7\frac{3}{4}$	1 to 32
No. 5 . . .	11	0	$6\frac{1}{8}$	1 to 29
No. 6 . . .	11	$15\frac{1}{2}$	$6\frac{1}{2}$	1 to $29\frac{1}{2}$
No. 7 . . .	15	$5\frac{1}{2}$	$8\frac{3}{4}$	1 to 28
No. 8 . . .	24	$4\frac{1}{2}$	$11\frac{3}{4}$	1 to 33
No. 9 . . .	14	$9\frac{1}{2}$	$7\frac{1}{8}$	1 to 32
No. 10 . . .	17	0	$8\frac{7}{8}$	1 to $30\frac{1}{2}$
No. 11 . . .	9	8	$7\frac{1}{4}$	1 to 21
Total	158	$11\frac{1}{2}$	$85\frac{1}{4}$	1 to 30

The average of these eleven examples of dogs, restricted for some days prior to death to an animal diet, thus gives to the liver a weight equal to the $\frac{1}{30}$th part of that of the animal. Stating it in other words, there is very nearly half an ounce of liver for every pound the animal weighs.

Looking to the amount of amyloid substance present in these livers, my analyses have yielded the following results:—In seven out of the eleven examples, examinations were made under precisely similar circumstances. The liver was removed and weighed immediately after death, and a piece at once taken for analysis, so as to avoid more loss than was possible from *post-mortem* transformation into sugar.

Amount of Amyloid Substance in the Liver of the Dog after a Diet of Animal Food.

	per cent.
Example No. 3	8·29
Example No. 4	5·24
Example No. 7	5·61
Example No. 8	8·45
Example No. 9	4·88
Example No. 10	10·95
Example No. 11	6·94

Average amount of amyloid substance yielded by the above seven analyses 7·19 per cent.

The excluded examples are thus accounted for. In example No. 1 no analysis was made. In No. 2 the liver was left in the animal for $2\frac{1}{2}$ hours before it was examined.

It then yielded 3·37 per cent. of amyloid substance. In No. 5 the liver was left in the animal for ten minutes and then removed, but not examined for the space of two hours. It then yielded 3·51 per cent. of amyloid substance. In No. 6 an error occurred in the steps of the analysis, rendering it valueless.

The usual allowance for a dog in my laboratory is one of the ordinary penny bundles of tripe *per diem*. In examples Nos. 7 and 8 an extra allowance was given, to see if it occasioned any difference. To No. 7 was given two bundles of tripe daily for four days prior to its death. No. 8 was allowed its full tether, and the quantity of tripe it devoured was truly enormous; three bundles one day, four another, five the third, and two and a half the day it was killed. The figures yielded do not disclose any significant variation from the ordinary condition.

To ascertain the effect of a vegetable diet on the liver, I submitted five dogs to a course of food for several days previous to death, consisting of barley meal and potatoes, or, where this was refused, to bread and potatoes. The following are the results obtained:—

The Liver of the Dog under a Diet of Vegetable Food.

	Weight of dog. lbs.	oz.	Absolute weight of liver. oz.	Relative weight of liver to animal.
No. 12	17	8	$19\frac{1}{4}$	1 to $14\frac{1}{2}$
No. 13	11	8	$12\frac{1}{2}$	1 to $14\frac{1}{2}$
No. 14	15	8	$11\frac{3}{4}$	1 to 21
No. 15	18	10	28	1 to $10\frac{1}{2}$
No. 16	17	5	$12\frac{1}{4}$	1 to $22\frac{1}{2}$
Total . . .	80	7	$83\frac{3}{4}$	1 to 15

Taking the average of these five instances as a sample of the effect of a vegetable diet on the liver of the dog, it appears that the organ rather more than equals in ounces the number of pounds the animal weighs. It will be remembered that, after an animal diet, the average given was rather under the half ounce to the pound. The facts as to the amount of amyloid substance stand thus:—

No analyses were made of the livers of the dogs Nos. 12 and 13, but the quantity of amyloid substance was unusually large. It was whilst examining these livers for the determination of sugar that I was first led to notice the effect of a vegetable diet that I am now describing.

Amount of Amyloid Substance in the Liver of the Dog after a Diet of Vegetable Food.

	per cent.
Example No. 14	9·87*
Example No. 15	25·30
Example No. 16	16·50

Average amount of amyloid substance yielded by the above three analyses 17·23 per cent.

* This liver not examined till one and a half hour after death.

The effect of giving an admixture of sugar with animal food is similar to that produced by a vegetable diet. The sugar employed in my experiments was the brown or moist sugar that is used for domestic purposes. Various devices had to be resorted to to get the animal to take it. The plan I found to succeed the best was to introduce it into short lengths of the small intestine forming part of the bundle of tripe. I will give the leading particulars belonging to each of the four dogs that formed the subjects of experiment in this way.

No. 17. A nearly full-grown mongrel dog, kept for eight days on a diet consisting of sugar and a bundle of tripe *per diem*. At first one-third of a pound of sugar was administered daily, but after three or four days the animal showed a disinclination for food, vomited, and had bilious diarrhœa. The quantity of sugar was reduced to a quarter of a pound daily. The dog now devoured voraciously all that was given to it. The urine collected from the bladder after death gave a strong reaction of sugar (grape-sugar).

No. 18. A youngish dog, fed for nine days on a bundle of tripe and a quarter of a pound of sugar daily. It consumed its food well at first, but during the last few days a great amount of coaxing was required to get it to take its full allowance. There was scarcely any urine to be procured from the bladder after death; what there was gave no saccharine reaction.

No. 19. A middle-aged dog, kept for eight days on a bundle of tripe and a quarter of a pound of sugar daily. The urine at death gave a slight but decided reaction of sugar.

No. 20. A dog not quite full-grown. Fed for five days on the same diet as the preceding dog. The urine collected after death gave a strong reaction of sugar.

The Liver of the Dog after a Diet of Animal Food with an Admixture of Sugar.

	Weight of dog.		Absolute weight of liver.	Relative weight of liver to animal.
	lbs.	oz.	oz.	
Example No. 17	10	3	12	1 to $13\frac{1}{2}$
Example No. 18	11	14	$12\frac{3}{4}$	1 to $14\frac{1}{2}$
Example No. 19	17	11	$10\frac{3}{4}$	1 to 26
Example No. 20	12	0	$13\frac{1}{2}$	1 to 14
Total	51	12	49	1 to $16\frac{1}{2}$

The average yielded by these four dogs gives a relative weight of liver bearing a close approximation to that after a diet of vegetable food; in the one case being as 1 to $16\frac{1}{2}$, in the other as 1 to 15. The amount of amyloid substance in each case was large, as is shown by the following results of analysis:—

Amount of Amyloid Substance in the Liver of the Dog under a Diet of Animal Food and Sugar.

	per cent.
Example No. 17	12·80
Example No. 18	17·55
Example No. 19	12·33
Example No. 20	15·37

Average amount of amyloid substance yielded by the above four analyses 14·5 per cent.

The relative weight of the liver and the amount of amyloid substance present have varied much in the observations that I have made on the rabbit. But the better the condition of the animal, the larger is the quantity of amyloid substance its liver contains. In the two following experiments, the effect produced on the liver by a free administration of sugar and starch is strikingly corroborative of the results obtained upon the dog.

In one of the experiments, a couple of full-grown rabbits were taken as near as possible resembling each other. One was kept entirely without food; the other was fed daily for three days, through a tube passed down the œsophagus into the stomach, with one ounce of starch and three-quarters of an ounce of grape-sugar made into a semifluid mass with water. On the fourth day both animals were killed.

	Weight of animal. lbs. oz.	Weight of liver. oz.	Amount of amyloid substance. per cent.
Rabbit fasting	3 1	$1\frac{2}{5}$	1·3
Rabbit on starch and grape-sugar . .	3 4	$2\frac{4}{5}$	15·4

The other experiment was on two half-grown rabbits, likewise as nearly as possible resembling each other. One was made to fast, whilst the other was fed on an ounce of starch and the same quantity of cane-sugar daily for three days. On the fourth day the examination was made.

	Weight of animal. lbs. oz.	Weight of liver. oz.	Amount of amyloid substance. per cent.
Rabbit fasting	1 14	1	1·4
Rabbit on starch and cane-sugar . .	1 $14\frac{3}{4}$	$2\frac{3}{8}$	16·9

I have a record of another experiment, but in this the amount of amyloid substance was all that was determined. The rabbit was allowed to take its ordinary food, and in addition, three-quarters of an ounce of loaf-sugar and half an ounce of starch were administered daily for three days. The animal was killed on the fourth day. The liver was not analysed until the day after death, but it then yielded 22·7 per cent. of amyloid substance.

After the administration of sugar, as in the above experiments, the liver is altered in its physical appearance. It becomes of a very pale colour, and so soft that it is readily

broken down by slight pressure between the fingers. I have seen it indeed so softened as to be almost pulpy, scarcely holding together when taken up by a pair of forceps.

I have made some analyses to show the relation that exists, in the transformation of amyloid substance into sugar in the liver after death, between the loss of the one and the gain of the other—how much sugar is formed for the hepatine that disappears. In the first three experiments, a part of the liver was instantly after death thrown into a freezing mixture, whilst the other was allowed to remain in the animal for some minutes. An analysis of the two specimens gave me the quantity of amyloid substance lost in the portion where the temperature was kept up, and likewise the amount of sugar that was formed. In the fourth, the liver was simply removed from the animal and examined at once, and then again in twenty-four hours' time.

	Amount of amyloid substance lost. per cent.	Amount of sugar gained. per cent.	Relation of gain of sugar to loss of amyloid substance.
No. 1	2·20	1·57	1 to 1·40
No. 2	7·04	4·25	1 to 1·65
No. 3	3·12	2·05	1 to 1·52
No. 4	1·82	1·12	1 to 1·62

Looking at the medium of these results, we get for the production of one part of sugar a loss of 1·54 of amyloid substance.

The following is a summary of the conclusions arrived at in this communication:—

The deductions drawn from an examination of the blood after death, without the observance of certain precautions, are fallacious. Blood collected ordinarily from the right side of the heart, after the destruction of life in a healthy animal, is strongly charged with sugar. This is not the condition naturally belonging to life; for if blood be drawn through a catheter from the right ventricle of a living animal which has remained in a quiescent state during the operation, it gives only a mere trace of the presence of sugar.

On suddenly destroying life, and instantly collecting the blood contained in the right side of the heart, it is found as free from sugar as if it had been collected during life.

From some recent carefully conducted experiments, the blood of the right side of the heart of an animal feeder is not distinguishable, as regards the amount of sugar present, from the blood of the portal vein.

Obstruction of the breathing determines an unnatural increase of sugar in the circulation, and a strongly diabetic state of the urine may be induced in this way.

The physiological or *ante-mortem* state of the liver, like that of the blood flowing from it, has been erroneously inferred, from the *post-mortem* condition that is presented. The liver at the moment of death is free, or almost completely free, from sugar, and the saccharine state which has been hitherto considered as belonging to it naturally during life, is in reality due to an easily recognizable chemical transformation which takes place with an astonishing rapidity after death.

The material existing in the liver, which is the source of the production of the sugar, is itself formed from sugar and its ally, starch. Dogs fed upon a diet abounding in starchy and saccharine materials have relatively very much larger livers, and livers strikingly richer in amyloid substance, than dogs fed upon a purely animal diet. In the rabbit fasting, the liver is exceedingly small and devoid of amyloid substance; whilst in the rabbit kept on starch and sugar only, the liver is large and exceedingly rich in amyloid substance.

In the transformation of amyloid substance into sugar in the liver after death, the production of one part of sugar is accompanied with the loss of 1·54 of amyloid substance as the average of four analyses.