Cameron Prize Lectures

ON

THE NATURE OF CONTROL OF THE METABOLISM OF CARBOHYDRATES IN THE ANIMAL BODY.

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BY

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ALLOW me, first of all, briefly to outline the general nature of our problem. Carbohydrates in their metabolism in the animal body are gradually broken down into smaller molecules which are finally oxidized to yield CO_2 and H_2O . They are absorbed, mainly as glucose, and transported by the blood in this form to the tissues in which their oxidation occurs. A large part of the glucose, if indeed not all of it, is, however, condensed into glycogen before the final breakdown process, one purpose of the formation of this substance being that the glucose may be stored, mainly in the liver, until it is required for the production of energy.

During starvation glucose is derived from glycogen until the stores of this substance are exhausted, when new glucose is produced out of protein and, possibly, fat. Life, apparently, is impossible in the absence of glucose, for it is present in the blood of the higher animals up to the time of death by starvation, and it occurs in the blood, or corresponding circulating fluid, of every form of animal in which this can be obtained for analysis. Not only must glucose be constantly supplied by the blood to the tissues, but this supply must vary greatly at different times according to their activity.

There are therefore two distinct and separate problems which require attention: first, the control of the supply of glucose to the tissues, or its mobilization, as it is sometimes called; and, secondly, that of its utilization.

An estimate of the rate of utilization of carbohydrate by the animal may be obtained by studying the respiratory exchange and by comparing the intake with the output of sugar. The rate of supply of glucose to the tissues could be directly measured in the animal as a whole only by comparing the percentages of this sugar in the arterial and venous blood at known velocities of blood flow, but for practical reasons it is impossible to obtain the data necessary for this measurement. The percentage of sugar in the venous blood—as is used clinically—serves only as an index of the balance between the rates of supply and utilization, but it is nevertheless an important value to determine; since, when taken along with estimates of the rate of utilization, it is an indirect measure of the rate of supply. The remarkable constancy of the blood sugar in health shows that there must be a very finely adjusted co-ordination between the two processes, and the question arises as to how this is effected. At one time it was thought to be through nervous reflex action, but as evidence has accumulated this view has very largely given way to one in which chemical substances present in the blood are believed to be the controlling factors. These substances are supposed to act as chemical messengers, or hormones, between the places in the body where glucose is being used up and those from which new supplies can be derived. The percentage of glucose in the blood cannot itself act in this way—although, no doubt, it is a most important factor—since the co-ordination breaks down in diabetes. The presence of some other agency is necessary, and it must be to the absence of this that diabetes is due.

It is now well known that diabetes is associated with disease of the pancreas, and evidence has gradually accumulated to show that this depends on the fact that the gland produces an internal secretion whose presence in the blood is necessary in order that changes in the concentration of sugar in the blood may serve to control its rate of production in the liver, out of glycogen or protein. When this internal secretion is absent, not only does utilization of

glucose in the tissues become seriously interfered with, but the glucose which is absorbed during digestion fails to be stored as glycogen, and the organism cannot use the glucose which is split off from protein.

Although these facts have been known for many years it is only recently that it has been possible to isolate from the pancreas the actual hormone to which this gland owes its controlling influence. The first step in this direction was taken by Banting and Best, who showed that the hormone could be extracted from the pancreas after the cells which produce the strong digestive enzymes had been caused to degenerate by previous ligation of the ducts. The next step was to seek for some extractive for the intact gland, in which the digestive enzymes would be incapable of attacking the hormone, and acid alcohol was found to fulfil these conditions. And the final step, due mainly to the work of J. B. Collip, was to purify the alcoholic extracts sufficiently so that they could be given subcutaneously, or intravenously, to man without causing local irritation or general toxic reactions. This mode of administration is imperative in order to avoid destruction of the hormone by the digestive juices of the stomach and intestines. I need not remind you that the name "insulin," previously suggested by Sir Edward Sharpey Schafer, has been given to these purified extracts.

By studying the effects of insulin in the diabetic and normal animal it is possible to determine with much greater certainty than hitherto the exact nature of the control of carbohydrate metabolism, and I propose in these lectures to review the main results which have been obtained, and to indicate how the investigation of other problems of metabolism besides that of the carbohydrates may be advanced by studying the action of this hormone.

Since diabetes is a condition in which the regulation of carbohydrate metabolism has become upset, both in the utilization and mobilization of sugar, owing to the absence of insulin, it will be simplest if we start with a consideration of the effects which follow in the diabetic animal when this hormone is restored, as by its administration from without. We will then study the action of insulin on certain forms of so-called experimental diabetes in which, as it were, only one part of the process of sugar regulation breaks down—namely, the mobilization of sugar out of glycogen. Since several of these forms are clearly due to disturbances in the nervous control of the glycogenic function of the liver, we will be in a position to see to what extent insulin may act through an influence on this type of control. There is some evidence that in man an unbalanced state of the nervous system, due to inheritance or to nerve strain, is frequently associated with diabetes. In the light of the information gained from these investigations we will consider the effects of an excess of insulin in the normal animal. In doing this we will, of course, have to bear in mind that although insulin is necessary for the control of carbohydrate metabolism its amount in the normal animal is finely regulated to the requirements so that the introduction of an excess, as by injection, must upset this balance and thereby create a condition which may be just as abnormal as that resulting from absence of the hormone. Finally, it will be of interest to consider the source from which insulin is derived in the pancreas, and to see whether it is solely through the production of insulin that this gland owes its profound influence, not only on the metabolism of carbohydrates, but on that of fat and perhaps of protein as well.

EFFECTS OF INSULIN ON THE DIABETIC ORGANISM.

For the consideration of this subject we will make use mainly of the experimental form of the condition produced in laboratory animals by complete removal of the pancreas. The results obtained by such investigations have been shown to be exactly the same as those obtained in diabetes mellitus in man. It is obvious, however, that the observations on depancreatized animals are of much greater value from the scientific standpoint than those on diabetic patients—partly because they are not confused by the presence of some remnants of still diseased pancreas, and partly because the chemical changes which are regulated by insulin can be much more thoroughly investigated by analysis of the tissues after death.

The most important and the earliest symptom of diabetes is increase in the percentage of sugar in the blood. At first this may be evident only after food has been taken, but later it becomes permanent, so that blood removed in the morning before food has been taken also contains a high percentage of sugar. Hyperglycaemia, as the condition is called, may be regarded as the initial symptom of diabetes. In depancreatized dogs it soon becomes very marked, and it was by observing its variations that Banting and Best tested the effects of their extracts of partially degenerated pancreas; a decided lowering of blood sugar was found to occur. When the hyperglycaemia reaches a certain level some of the sugar escapes by the urine and the symptom of glycosuria becomes established. It was found that the extracts also diminished or removed this symptom—a result of great importance, since it indicated that the reduction of blood sugar could not depend on increased leakage of sugar through the kidneys, such as occurs in the form of experimental diabetes which is caused by phlorizin, but must depend on a change in the rate of production or utilization of sugar in the organism itself.

In the earlier experiments it was not possible to measure accurately the relation between the intake of sugar with the food and the output by the urine, but this has recently been done by F. N. Allen with results which are of great interest, both from a scientific and a practical standpoint, and which I wish to lay before you in some detail.

Depancreatized dogs fed on a constant diet of lean meat and cane sugar were kept alive by daily injections of insulin. They put on weight, remained free from any of the general symptoms of diabetes except a certain degree of glycosuria and occasional hyperglycaemia, owing to the relatively large amount of carbohydrate which was contained in the food. The particular problem of the investigation was the behaviour of the sugar balance when different doses of insulin were given, by which is meant the difference between the amount of glucose excreted and the total amount (derived both from preformed carbohydrates and from protein) ingested. If we compare this balance during different periods in which the dose of insulin is made to vary we can determine the glucose equivalent of insulin—that is, the number of grams of this sugar which each unit of insulin is capable of causing to be metabolized in the animal. It does not matter, for this purpose, what we take as the unit of insulin, so long as it is always the same, but for convenience we have chosen the so-called clinical unit, which is one-third of the amount which can lower the blood sugar of a rabbit to a certain level. By way of example, let us suppose that with the total daily glucose intake at 145 grams and with 20 units of insulin the output is 35 grams (giving a utilization of 110 grams), and that with 30 units the output falls to 5 grams (giving a utilization of 140 grams), then the 10 units have caused 30 extra grams of glucose to be metabolized, and the glucose equivalent of a unit is 3. Allen has found that this value becomes progressively less as the dose of insulin is increased, as is shown in the following table.

<i>Dog I. fed daily on 600 grams Lean Meat and 100 grams Sucrose.</i>									
Insulin given daily (clinical units).					Glucose equivalents (on different days).				
20	...	...	...	...	...	5.8	5.8		
24	...	...	...	...	...	4.1	4.0		
32	...	...	...	...	...	3.5	3.5	3.6	3.3
								3.7 (4.1)	
36	...	...	...	...	...	3.0	3.1	3.1	
38	...	...	...	...	...	3.4			
40	...	...	...	...	...	3.0	3.3	3.1	3.0

Same Dog fed on 600 grams Lean Meat but with varying Amounts of Sucrose.

A. Insulin given daily 20 units.

Sucrose given daily.					Glucose equivalents.				
50 grams	...	...	...	...	...	3.5	3.9		
100 "	...	...	...	...	...	5.8	5.8		
150 "	...	...	...	...	...	8.4	8.1	6.9	6.9

B. Insulin given daily 32 units.

50 grams	...	...	...	...	...	2.4	2.6		
100 "	...	...	...	...	...	3.7	4.1		
125 "	...	...	...	...	...	4.9	4.8		
150 "	...	...	...	...	...	4.5	4.9		

There are probably several reasons for the progressive decline in the glucose equivalents as the dosage of insulin

is increased. One of these no doubt is that when a relative excess of insulin is given some is either lost in the urine or destroyed. This cannot be the only factor, however, as is shown in the second part of the table in which the glucose equivalents are given for different periods during each of which the amount of insulin is constant but the amounts of sugar given daily are increased. This indicates that another factor comes into play and that the action of insulin in the control of glucose metabolism obeys the same laws as those which govern enzyme action. These state that when the substrate is large in relationship to the enzyme the latter develops its maximal effect and the amount of substrate attacked is almost directly proportional to the amount (that is, the number of units) of enzyme; but when, on the other hand, the amount of substrate is small in relationship to the amount of enzyme, the increasing amount attacked by each added unit of the enzyme becomes progressively less and less, the reaction proceeding according to a logarithmic curve. Dr. Allen has found the equation for the curve of the glucose equivalents of insulin, and it is significant that it is of the same type as that found by Dr. Murray Lyon for the pressor effect of adrenaline. The value of knowing this relationship is partly that it furnishes us with a method for assaying the strength of insulin, and partly that it shows that it is useless to try to measure the glucose equivalent of insulin on hospital patients without taking into consideration the glucose balance. Failure to do so is no doubt the cause for the varying results in this connexion which Wilder, Boothby, etc., and Woodyatt have obtained in the clinic. The results also suggest that it may be of value to use small doses of insulin in clinical cases in which for one reason or another a full dose cannot be given. If this should prove to be the case encouragement is given to see whether small amounts of insulin can be absorbed by other pathways of administration than that of subcutaneous injection.

One other point of significance is that the equivalent is apparently not the same for glucose derived from protein and that derived from preformed carbohydrates. Thus the lowest equivalent obtained in Allen's experiments is three, whereas that obtained under comparable conditions on patients receiving very little carbohydrate in their diet is seldom over two. Wilder, Boothby, etc., on other evidence have also concluded that glucose from the two sources is not metabolized with equal facility. A similar relationship between dose and effect no doubt obtains in the case of the lowering of blood sugar by insulin, but it has so far been impossible, in normal animals, to obtain sufficiently constant data from which to determine the curve. The varying extent to which new sugar is produced to replace that which has disappeared and individual variability in the susceptibility of animals to insulin are the chief causes for this inconstancy.

EFFECT OF INSULIN ON SYMPTOMS OF DIABETES OTHER THAN HYPERGLYCAEMIA AND GLYCOSURIA.

Besides hyperglycaemia and glycosuria, there are various other objective symptoms of diabetes which can be accurately measured in depancreatized dogs and upon all of which insulin has a profound influence. We will now review some of the results which illustrate this influence.

It will be remembered that glycogen stands at the one end of the chain of metabolic processes of carbohydrates with the oxidation products CO_2 and H_2O at the other, and it is significant that in diabetes glycogen disappears from the liver and oxidation fails to occur. Insulin is necessary for both the initial and the final stages of the process, as is evidenced by the fact that its administration to diabetic animals immediately restores both of them to normal. The following table shows the effect of insulin on glycogen formation.

Percentage of Glycogen in Depancreatized Dogs after Feeding with Sugar.

					Liver.	Heart.
A. Without insulin	...	...	...	...	0.047	0.85
"	"	...	...	...	0.34	—
"	"	...	...	...	1.30	0.98
B. Following insulin	...	...	...	...	20.0+	—
"	"	...	...	...	12.85	0.73
"	"	...	...	...	11.40	0.57
"	"	...	...	...	4.8	0.30

It is of interest that the first analysis for glycogen in these insulin-treated animals, made by J. B. Collip, actually gave higher results than have ever been recorded even for normal well fed animals. Evidence that the combustion process is also involved is afforded by studying the ratio between the volume of O_2 absorbed by the animal and that of CO_2 produced in the same time. In a healthy animal fed on carbohydrates these approach equality, but in a diabetic one the volume of O_2 exceeds that of CO_2 in the ratio of 1 to about 0.67. The respiratory quotient, as it is called (R.Q.), serves therefore as an indicator as to whether combustion of carbohydrates is proceeding normally. In a depancreatized dog in which the quotient stood at 0.67 and did not rise when large amounts of sugar were injected, insulin given along with the sugar caused it to rise to 0.89.

Fat Metabolism in Diabetes.

It is not only carbohydrate metabolism itself which goes out of order in diabetes, but also that of fats; and here again we have to deal with a long chain of chemical processes, starting with the large molecules of fatty acid, proceeding, by a gradual splitting off of methyl radicles, until a residue containing only four of them is left (butyric acid) and ending in an oxidation process in which β -oxybutyric acid, aceto-acetic acid (the ketone bodies), and finally CO_2 and H_2O are produced. Preceding the first stage in this complicated process the fat is apparently transferred from the fat depôts to the liver, where it is in some way prepared for the breakdown process, after which it is carried back to the tissues to be further dealt with. In diabetes not only does the transference of fat become upset, but the final oxidation process, beyond the butyric acid stages, fails to proceed smoothly, with the consequence that fat accumulates in the liver and blood, and ketone bodies appear in the blood and urine, causing the serious symptoms of coma, so well known in this disease. The following tables show the effect of insulin on both these stages in the metabolism of fats.

Percentage of Fatty Acid in Depancreatized Dogs after Feeding with Sugar.

				Liver.	Blood.
A. Without insulin	...	...	...	12.25	—
"	"	...	...	14.10	1.2
"	"	...	...	9.9	1.12
B. Following insulin	...	...	...	7.42	0.30
"	"	...	...	2.19	0.53
"	"	...	...	4.41	—

Daily Excretion of Sugar and Ketone Bodies in Depancreatized Dog.

Day.	Treatment.	Sugar.	Ketone Bodies.
1	No insulin	29.7 gm.	100 mg.
2	No insulin	28.4 "	187 "
3	Insulin	4.25 "	0 "
4	No insulin	10.00 "	0 "
5	"	9.60 "	0 "
6	"	25.2 "	34 "
7	"	25.4 "	55 "
8	"	18.0 "	114 "
9	Insulin	8.0 "	0 "

(J. B. Collip.)

In interpreting the action of insulin on the metabolism of fats one must not lose sight of the probability that it is a secondary one through its effect on the carbohydrates. At least in the final stages it is commonly believed that combustion of glucose is essential to the oxidation of fats. "Fats burn in the fire of carbohydrates," and insulin, by stirring up this fire, allows the fats to be consumed.

Besides these effects on what we may call the measurable symptoms of diabetes, insulin also greatly improves the general condition of the animal and prolongs its life. Thus Banting and Best succeeded in keeping a depancreatized dog alive for seventy days, as compared with about fourteen, which is the usual survival period for treated animals. In man the distressing subjective symptoms of the disease are also influenced in a most striking fashion. Unfortunately the animal observed by Banting and Best was not allowed to live until it could be seen for how long life might be pro-

longed by the use of insulin. This has more recently been done by Dr. F. N. Allen in the case of the same animals as were used for measuring the glucose equivalents of insulin. One of the animals has lived for over five months and two others for four months, when they succumbed to gastrointestinal symptoms, related no doubt to the entire absence of the pancreas. The cause of these symptoms is being investigated at present.

ACTION OF INSULIN ON GLYCOGENOLYSIS.

While these results with insulin on depancreatized animals show conclusively that the hormone affects all stages of carbohydrate metabolism, they do not indicate whether the action is primarily on the first or the last stages of the process. It becomes of interest, therefore, to investigate the action of insulin on those forms of hyperglycaemia which are due entirely to an upset of the first stage—namely, the conversion of glycogen into sugar—and which may be classified as nervous, asphyxial, and pharmacological according to the means used to produce them. Among the nervous forms of hyperglycaemia are Claude Bernard's famous piqure experiment, and stimulation of the sympathetic nerve supply of the liver, either electrically or by injecting adrenaline. The asphyxial forms include mechanical asphyxia and poisoning with carbon monoxide; and the pharmacological, the effect of anaesthetics and certain narcotics, though here also an asphyxial element is probably included. It is significant that insulin can entirely prevent the development of hyperglycaemia in all these forms. Most attention has been paid to adrenaline hyperglycaemia, partly because it is possible in this case, by grading the dosage, to produce varying degrees of increase in blood sugar and so to assay insulin in terms of the amount of adrenaline which it can antidote, and partly because adrenaline and insulin are both hormones.

The fact that insulin prevents hyperglycaemia in the experimental conditions does not in itself show that this hormone inhibits the glycogenolytic process, for, while this is proceeding at a supernormal rate, the excess of sugar which results may fail to raise the blood sugar, because of its removal, through the presence of insulin, as quickly as it is produced. There are two observations which have a bearing on this problem. When glucose is injected subcutaneously into a rabbit the curve of blood sugar rises rapidly to a certain height and remains elevated, depending on the amount injected. When the injections are made following insulin the curve is greatly reduced, both in height and duration, the most marked effect being obtained when the sugar is given in a little over an hour after the insulin (G. S. Eadie). Evidently, therefore, the insulin accelerates the rate at which the excess of glucose disappears. On the other hand, there is evidence to indicate that insulin also retards the glycogenolytic process, for, as E. C. Noble has shown, much more glycogen remains in the liver after repeated injections of adrenaline along with insulin than after injections of the same amounts of adrenaline alone. This is shown in the following table:

Retarding Influence of Insulin on the Glycogenolysis due to Adrenaline.

(Percentage of glycogen in livers of fed rabbits eight hours after injection of adrenaline alone, and of adrenaline plus insulin.)

Experiment 1.			Experiment 3.		
After Adrenaline.	After Adrenaline plus Insulin.		After Adrenaline.	After Adrenaline plus Insulin.	
1.40	...	8.12	2.30	...	3.34
1.96	...	6.40 C.	1.10	...	10.90
		3.64 C.	3.10		
Experiment 2.			Experiment 4.		
8.56	...	12.24	1.40	...	8.12
6.60	...	1.60 C.	1.96	...	6.40 C.
		5.60			3.64 C.

C. indicates that the animal showed convulsions.

A possible explanation for these apparently contradictory results is that insulin causes any excess of sugar in the blood to be converted into some non-reducing substance which by its accumulation prevents the breakdown of glycogen. This substance is certainly not glycogen itself, since this material, instead of becoming increased when

glucose is given along with insulin, actually becomes reduced, for reasons which will be explained later on.

Taking these observations on diabetic and hyperglycaemic animals as a whole it would appear that insulin is necessary for the conversion of glucose into some substance which is closely related to glycogen and the formation of which is also a necessary step in its preparation for final oxidation. Winter and Smith have supposed that this substance is related to the highly active form of glucose (γ -glucose) recently identified by Irvine and his school, but the evidence which they offer is by no means convincing.

EFFECT OF INSULIN ON NORMAL ANIMALS.

Turning now to the effect of insulin on normal animals we may start with the observation, first made by J. B. Collip, that the blood sugar also becomes lowered, and, if the dose of insulin be sufficient, may almost disappear. This hypoglycaemia persists for a period the length of which depends partly on the amount of insulin injected and partly on the nutritional condition of the animal. If we compare curves of the blood sugar in different rabbits injected with varying quantities of insulin, it will be observed that during the half-hour or so following the injection the curves all descend at approximately the same rate and that it is difficult to make out any close relationship between the rate of fall and the dose. Neither has the amount of glycogen in the liver of the injected animals any influence on the initial fall. It is just as rapid in animals with over 10 per cent. of glycogen in the liver as in those that have none at all. After this initial stage, however, the curves vary considerably, those from animals with large stores of glycogen tending to recover the normal level much more rapidly than those from animals that are aglycogenic. The influence of varying amounts of glycogen on the rate of recovery of blood sugar can be eliminated by using rabbits in which the stores of this material have been reduced to a minimum by starvation, and under these conditions the degree of hypoglycaemia can be used as a measure of the strength of the insulin injected, this forming the basis of the present method for its physiological assay. Evidently the hypoglycaemic condition stimulates the mobilization of fresh glucose out of the glycogen stores, and it is significant, as Burn has shown, that this mobilization does not occur if the nerve supply of the liver be cut off, as by a paralysis of the nerve terminations by means of ergotamine. This result has an important bearing in connexion with the general problem of the nature of control of the metabolism of the carbohydrates, for it shows that nerve reflex action is a factor which acts along with insulin. I need scarcely point to the practical importance of these results in connexion with the administration of insulin in medical practice. When it is given to diabetic patients for the first time the blood sugar may become lowered to a dangerous level because of the poverty of the stores of glycogen.

Nervous Hyper-irritability.

When the blood sugar has reached a level of about 0.045 per cent. symptoms of a peculiar nature supervene. These vary somewhat in different animals, but in general may be described as consisting of a state of nervous hyper-irritability often accompanied by muscular tremors, evidence of hunger, rapid breathing, stimulation of the salivary and sweat glands, and pupillary effects. In many animals these more or less premonitory symptoms are followed by convulsions in which the animal may throw itself about in a violent fashion. The convulsive seizures last for a few minutes and the animal then lies in a collapsed state with quickly falling body temperature and rapid shallow breathing, until another convulsive seizure supervenes. These two main symptoms repeat themselves alternately, the coma becoming more and more pronounced and the convulsions feebler until at last the animal dies of respiratory failure, the arterial blood being intensely dark in colour and the muscles instantly passing into rigor mortis. We have no time here to discuss the underlying cause for the symptoms, but it is of great significance that they are instantly removed, even after they have been present for some time, by the injection of glucose. The symptoms are evidently

closely related to the disappearance of free glucose in the nerve cells, particularly those of the medulla and neighbouring portions of the pons. No other sugar, not even laevulose, mannose, or galactose, which are so closely related to glucose, has anything like the antidoting power that glucose has. Since the symptoms are liable to occur in fed animals when the blood sugar is about 0.045 per cent. it has been convenient to choose this as the level to which one unit of insulin should lower it.

The Cause of the Decrease of Sugar in Normal Animals.

The question as to the cause of the decrease in the blood sugar in normal animals is proving a very difficult one to answer. The rapid character of the initial fall in blood sugar might suggest that it is dependent on some process occurring in the blood itself—to increased glycolysis—but that this is not the case has been shown by studying the rate at which the sugar disappears from blood during its incubation under sterile conditions outside the body. The addition of insulin to the blood does not influence the rate of glycolysis, neither is this different in normal blood and in that removed from animals injected with insulin at varying periods before bleeding. Evidently the sugar disappears from the blood by passing into the tissues, a conclusion which is confirmed by observations on the rate of disappearance of glucose from saline solutions perfused through the mammalian heart. Insulin must, therefore, cause a lowering of the concentration of sugar in the tissues, a sort of vacuum for sugar, so that sugar is removed from the blood to make good this loss. Why, then, does sugar disappear in the tissues? In diabetic animals it is quite clear, as we have seen, that the sugar disappears partly because it is converted into glycogen and partly because it is oxidized to CO_2 and H_2O , and it might be supposed that the same processes would be responsible for its disappearance in normal animals as well. But recent investigations by McCormick, O'Brien, Noble, etc., show conclusively that increased glycogen formation does not occur, either in the normal rabbit or in the perfused liver of the turtle, when insulin is given. The possibility that increased oxidation of glucose is responsible has been investigated by observing the respiratory quotient and the energy balance. The results have so far been somewhat contradictory in character, but taking them as a whole, I think there is no doubt that the respiratory quotient becomes raised soon after insulin is given, indicating probably a relative increase in the oxidation of carbohydrates. This rise is neither sufficiently marked nor sufficiently sustained, however, to make it clear that increased combustion is the only cause for the disappearance of the sugar from the blood, especially when it is remembered that we must account not only for the sugar originally present but also for that derived from the glycogen, which, as Dudley, Trevan, etc., have shown, is broken down when insulin is given to well fed animals. It seems justifiable to conclude that insulin acts mainly by converting glucose into some substance which is a preliminary stage in its conversion into both glycogen and the breakdown products which are ultimately oxidized. Attempts to detect the presence of this substance in the tissues of animals injected with insulin (and sugar) have, so far, failed, probably because it has no reducing properties, upon which estimation of sugar itself depends, and because it is destroyed by the strong alkali which is used for the isolation of glycogen. That such intermediary substances of carbohydrate metabolism may exist has been suspected for long, because of the impossibility, after injecting large amounts of sugar into animals, of accounting for more than between one-half and three-fourths of the injected sugar in the entire animal, taking into account glycogen and free sugar. It is possible that this intermediary substance may be some complex containing phosphoric acid, since the free phosphates of the blood have been shown by Winter and Smith to become reduced in amount after injection of insulin. Whether or not such a substance is formed, there is evidence that the metabolism of protein becomes diminished by insulin, both in normal and diabetic animals (F. N. Allen), which would seem to indicate a protein-sparing action due to an increased metabolism of carbohydrates.

THE SEAT OF PRODUCTION OF INSULIN IN THE PANCREAS.

Finally, allow me to say a few words with regard to the seat of production of insulin in the pancreas. As the name implies, this is believed to be the cells of the islets of Langerhans, but, until the work to which I shall refer was completed, no direct evidence existed for the belief. The anatomists, particularly Laguesse, Diamare, and F. R. Bensley, have shown that the islets are structures composed of epithelial cells containing granules which are differentiated from those present in the acini by peculiar staining reactions. Lane, working with Bensley, has described two kinds of cells, one in which the granules are soluble in alcohol (beta cells) and another in which they are insoluble in alcohol (gamma cells), the two kinds also staining differently with neutral gentian violet. Islets cannot be differentiated from exhausted acini with certainty unless the cells give these characteristic microchemical reactions.

In diabetes in man the beta cells are frequently found to be destroyed or to be the seat of hydropic degeneration, but it is seldom that the pancreas can be obtained soon enough after death to permit of satisfactory differential staining. Often, however, the destruction is so extreme in degree that a large number of the islets are found to have disappeared entirely. These observations indicate the importance of having some method by which the number of islets can be counted, and, since this cannot be done with any degree of accuracy in ordinary sections, Bensley has introduced so-called *intra vitam* methods in which dye-stuffs (Janus green or neutral red) are injected into the aorta of the animal immediately after death. The islets then stain differently from the remainder of the pancreas, and can be actually counted in weighed small pieces of pancreas pressed out under a cover-slip. The counts show not only that there is considerably more islet tissue than had hitherto been supposed to be the case, but also that the amount is fairly constant in such conditions as after starvation and after exhaustion of the secretory acini, which had been thought, by previous workers, to cause it to increase.

The majority of observers agree that the islets are structures that are independent of the acini, although Laguesse still maintains that, under certain conditions, they may be derived from this source. There is almost incontrovertible evidence, however, that this is not the case, but that they are derived from the duct epithelium, being directly connected with this in many of the lower vertebrates, such as the cartilaginous fishes (skate, dogfish) and indirectly in the mammalia, by fine epithelial tubules. Thanks again to Bensley, methods of *intra vitam* staining have been discovered by which these connecting tubules can be very clearly demonstrated, a fact of great importance in connexion with the question as to a possible regeneration of islet tissue after its destruction by disease, or by ligation of the secretory ducts of the pancreas.

The Islets in the Lower Vertebrates.

These facts make it of practical importance to study the relation of the islets in the pancreas of the lower vertebrates. As has already been pointed out the islets in the cartilaginous fishes are closely attached to the ducts, but a still more interesting fact is that in certain of the bony fishes (the angler fish, the sculpin, the flounder, halibut, the cod, and pollock) the islets actually exist distinct and apart from the acinar tissue, being no doubt connected with outgrowths from the ducts, although these connexions are difficult to see. The exact position of the islets in these fishes has been very carefully described by Rennie, following the work particularly of Diamare, and Dr. Slater Jackson of McGill University has recently informed me that he has succeeded in differentiating the alpha and beta cells in the islets. In the angler fish, for example, a very large islet, called the principal islet, exists in the mesentery lying between the spleen and gall bladder, and there is relatively very little acinar tissue associated with it. Time will not permit me to go into these anatomical details any further here, but sufficient has been said to indicate the important

bearing which a knowledge of them has on the question under discussion—namely, the source of insulin. Does it come from the islets or from the acinar tissue? By studying the effects of extracts prepared separately from islets and acinar tissue, it has been found that extremely potent insulin is present in the former, but not a trace in the latter. Indeed, an extract of acinar tissue free from islets, instead of causing the blood sugar to become lowered, actually causes it to be raised. There can be no doubt, therefore, that the islets are the only source of insulin in the vertebrate pancreas.

Quite apart from the theoretical interest of these results they raise the practical question as to whether fish may not prove to be a fruitful source for insulin. During the present summer my two collaborators, N. A. McCormick and E. C. Noble, have been engaged at the Atlantic Laboratory of the Biological Board of Canada at St. Andrews, New Brunswick, in investigating this possibility, with results which are decidedly encouraging. They have found, for example, that the principal islets of the cod are very readily accessible and can be easily recognized and removed by fishermen. They made arrangements for the collection of large quantities of these islets on the trawlers at Halifax, and have been able, by a very simple method of extraction, to prepare insulin of a degree of purity suitable for clinical use. The best yields have been from cod and halibut, which, since they are fish caught in large numbers, should therefore prove to be a profitable source of supply in this country. The yield of insulin per gram of islet tissue taken from the cod is about 25 units, or, stated in terms of the weight of the entire fish, about 1 unit per 5 lb. of fish; in another report they give 1 unit per 4 lb. of fish. This work is not yet completed, and McCormick and Noble are endeavouring, with the aid of the Biological Board of Canada and the National Fish Co., to work out the details of large scale production from these sources. In places where it is difficult to obtain slaughter-house material, it is possible that it would be economical to prepare insulin from fish.

The Islet and Acinar Tissue after Ligation of Ducts.

And finally, in light of the great interest which attaches to the original experiments of Banting and Best, by which they succeeded in demonstrating the presence of insulin in the duct-ligated pancreas, let us see, by using Bensley's *intra vitam* methods of staining, how the islet and acinar tissue respectively behave when the ducts are tied. It has been found that both types of tissue degenerate shortly after the operation, the acinar more rapidly and more profoundly than the insular. Both tissues then become regenerated, to degenerate again at a later period, and these processes of degeneration and regeneration go on for many months, regeneration of the islets becoming gradually more and more prominent, until finally nothing is left of the pancreas but fatty tissue with regenerated islets embedded in it. During all this time the animal remains free from the symptoms of diabetes. Of great significance is the fact that whereas the islets in the normal gland are scattered in the acinar tissue, with no evident duct connexions visible in ordinary sections of the gland, they become definitely grouped around and attached to the main ducts in the regenerated tissue, reverting therefore to a primitive type, such as is seen in the pancreas of the skate. The remarkable regenerative power of the islet tissue from duct epithelium, which these observations demonstrate, makes it seem probable that a similar regeneration will occur in the pancreas in diabetes in man. The aim of treatment in this disease should therefore be directed to resting the islets so that their regeneration may occur, and this can be accomplished by relieving them of the strain of producing insulin, either by greatly cutting down the carbohydrates of the food or by administration of extracts containing this hormone. Since it is probable that the condition which acts as stimulus to the production of insulin by the islets is the level of the blood sugar, the aim in diabetic therapy should be to keep this within the normal limits.