A Lecture ON INSULIN.

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SPEAKING for my collaborators, as well as myself, I wish to assure you that we consider it a very great honour that an account of our researches on insulin should be given so prominent a place on the programme of the XIth International Congress of Physiologists. In accepting the invitation to address you on this work, I fully realize the great responsibility that I have assumed, the more so since at the present time there yet remains so much that is obscure and unfinished, particularly with regard to the physiological aspects of the subject.

There is no other problem in the whole vast field of medical science that has attracted such diverse groups of workers as that of the relationship between the pancreas and disturbances in carbohydrate metabolism. Anatomists, pathologists, biochemists, physiologists, and physicians have contributed much to it, each adding some new and fundamental fact, and the knowledge thus acquired has formed the foundation upon which the present investigations depend. I shall not occupy your time here with a review of this earlier work except in so far as it is essential to an understanding of its relationship to these investigations.

HISTORICAL.

Towards the end of the seventeenth century Brunner, in his celebrated researches on the digestive functions of the pancreas, had occasion to remove the gland, and he described symptoms in the operated animals which were without doubt those of diabetes. This was, however, some time before the disease had come to be recognized as a clinical entity, and it was not until considerably later that physicians began to point out the frequent occurrence of morbid changes in the pancreas of patients who had died of it. The clue which this observation offered suggested to experimentalists, among them Claude Bernard, the advisability of seeing whether removal or destruction of the pancreas might cause diabetes. The experiments were without success until 1885, when von Mering and Minkowski announced their well known discovery, as a result of which Minkowski subsequently published a complete account of the pathological chemistry of the diabetic condition as observed in depancreated animals. As the cause of the diabetes Minkowski suggested the removal from the organism of "a something" that is essential for the metabolism of carbohydrates. He clearly meant an internal secretion, although it is in the writings of Lépine that for the first time we find this term actually used in connexion with the pancreas. But the existence of an internal pancreatic secretion could not be considered as an established fact until it could be shown that the administration of an extract of the gland to depancreated animals is capable of removing the diabetic symptoms, and this none of the investigators of this period could succeed in doing. These failures led some to suppose that the pancreas must control the metabolism of carbohydrate otherwise than by an internal secretion, although the majority considered that it was merely owing to destruction of the active principle during the preparation of the extracts that the failure to obtain it in extracts was due.

At the same time as these experimental investigations were in progress most valuable work was being done on the minute anatomy of the pancreas, particularly with regard to the islets of cells which had been described in 1869 by Langerhans as distinct and separate from the secreting acini. Among these workers I cannot refrain from paying tribute to Laguesse and Diamare and, among

more recent workers, to R. R. Bensley, and their respective collaborators. This work established the essential independence of the cells of the islets and acini, and, since the latter were obviously concerned in the production of the digestive secretion of the gland, it was postulated that those of the islets must be responsible for the internal secretion that controls the metabolism of sugar. This hypothesis was greatly strengthened by experimental and pathological evidence. Minkowski and Hédon and, later, W. G. Macallum, demonstrated that when a small portion of the pancreas is transplanted under the skin, the main gland can be completely removed without any symptoms of diabetes supervening. Schultze and Ssobolew, independently, found that complete blockage of the secreting ducts is followed by a degeneration which involves the cells of the acini, but not those of the islets. This was more or less confirmed by other investigators, and furnished strong support for the islet hypothesis, since no diabetic symptoms were observed in the operated animals. Finally, pathologists, particularly Opie, described definite histological changes in the islet cells in patients dying of diabetes mellitus.

But all this evidence, strong though it might be, could not be considered as affording positive proof that the pancreas produces an internal secretion necessary for the control of the metabolism of sugar, and renewed attempts to demonstrate its presence in pancreatic extracts did not meet with sufficient success to prove convincing. That this would ultimately be achieved, however, was apparent to many, and to none more so than to the President of this Congress, Sir E. Sharpey Schafer, who, by suggesting the name Insulin for the hormone, indicated his belief that its source is the isles of Langerhans.

The most successful attempts to prepare pancreatic extracts containing insulin were made by Zuelzer, who, in 1908, showed that alcohol extracted from pancreas which had previously been subjected to a certain degree of change by ligation of its veins, a substance which was capable, when injected subcutaneously, of preventing the rise in blood sugar and the glycosuria which ordinarily follows injection of epinephrin. Zuelzer carried his investigations far enough to show that alcoholic extracts of beef pancreas could also remove many of the symptoms of diabetes in man, but, unfortunately, he failed to establish the therapeutic value of his product because of certain toxic symptoms which followed its administration. It is possible that these may have been due, in part at least, to an excessive lowering of the blood sugar which we now know to be associated with peculiar symptoms. From this time on the problem of attempting to produce extracts of the pancreas containing insulin fell to the lot of experimentalists, among whom may be mentioned Knowlton and Starling in this country, and E. L. Scott, J. R. Murlin, and Cramer and I. Kleiner in America, but with only occasional success.

RECENT RESEARCHES.

We may take up the recent researches on insulin at this point; they had their origin in a suggestion, made by F. G. Banting, that since it was probable that the failure to preserve the active hormone in extracts of pancreas was due to the presence in them of proteolytic enzymes which would destroy it, the action of these might be circumvented by extracting glands that had been caused to undergo degeneration by previous ligation of the ducts. With the collaboration of C. H. Best, Banting ligated the ducts of the pancreas in several dogs, and a few weeks afterwards killed the animals by chloroform, quickly excised the partially degenerated residue of the gland, and extracted it with ice-cold Ringer's solution by grinding in a mortar with sand. Intravenous injection of the extract into depancreated animals was found to cause prompt and decided lowering of blood sugar, accompanied by a fall in the sugar excreted in the urine without any change in the percentage of haemoglobin in the blood. That the treated animal had regained some of its lost power to utilize carbohydrates was demonstrated by comparing the sugar balance, following administration of a measured amount of active sugar, with and without the extract. Incubation of active extract

with trypsin in alkaline reaction was found to remove its hypoglycaemic action, which was not the case when faintly acid extract was incubated without trypsin. Similarly prepared extracts of spleen and liver had no effect, neither had otherwise active extracts of pancreas when given by mouth or by rectum.

These results, confirmed many times, seemed sufficient to prove the existence of the elusive pancreatic hormone, and the next problem was to devise a method by which it could be extracted from pancreas obtained from the abattoirs. As a step to this, Banting and Best took advantage of the fact, established by Ibrahim, that up to four months of foetal life in the ox the pancreas does not contain active proteolytic enzyme, although there is evidence, furnished by the experiments of Carlson and Drennan, that the anti-diabetic hormone is produced by the gland during foetal life. They found that active extracts could be prepared from the pancreas of the foetal calf, and they carried the search with success to adult pancreas, using alcohol, as had previously been suggested by E. L. Scott and Zuelzer, as the extraction fluid, in the hope that in this medium proteolytic destruction of the hormone would not take place. Larger quantities of extract being now available, they succeeded in keeping a depancreated dog more or less constantly under the influence of the extract for a period of over two months, during which the animal gained in weight and exhibited only in slight degree the characteristic general symptoms of diabetes.

The criticism has been made that Banting and Best's experiment in which simple extracts of duct-ligated pancreas were used formed no essential step in the investigations which have given us insulin. I need scarcely reply to these criticisms. They were apparently made without any appreciation of the real obstacle that stood in the way of development of the subject—namely, convincing evidence that an antidiabetic hormone does actually exist in the pancreas—and to Banting and Best is due the credit of furnishing this by experiments of a different type from those of their predecessors. We owe much to the initiative, skill, and patience they displayed in completing this first essential step in the investigation.

It now became apparent that attempts should be made to see whether the crude alcoholic extracts of ox pancreas could be sufficiently freed from those substances which made them unsuitable for subcutaneous administration to diabetic patients, either because of local irritation or general toxic effects. At this stage of the investigation we were joined by J. B. Collip, who, in a remarkably short time, succeeded in isolating from the alcoholic extracts, by fractional precipitation with alcohol, a precipitate which contained the antidiabetic hormone in high concentration, and which, in watery solution, could be injected subcutaneously in man without any deleterious effects. At the same time it was also discovered that the blood sugar is lowered in normal rabbits by subcutaneous injection of extracts containing the antidiabetic hormone, and to this observation was due in large part the rapid progress which it was possible to make in the isolation of insulin. It offered a ready means for testing the relative potency of the various precipitates and filtrates, and of determining the most favourable conditions under which extraction of the gland should be carried out.

Provided with a comparatively simple method for the preparation of insulin, it now became possible to undertake a systematic investigation of its physiological and chemical properties and its possible therapeutic value, and I shall endeavour to describe briefly what has been found, and to indicate, in a general way, what appear to be the most hopeful lines for further research. It seemed advisable at this stage to group the problems demanding immediate investigation, and this was done as follows:

1. The effect of insulin on the respiratory exchange, the distribution of glycogen, and the metabolism of fat in animals rendered diabetic by pancreatectomy.
2. Its therapeutic effect in diabetes mellitus.
3. Its effect on the blood sugar of normal animals and the symptoms which result from overdosage.
4. Its pharmacological assay.
5. Its effect on the blood sugar in the various forms of experimental hyperglycaemia.

6. The physiological mechanism by which it lowers the blood sugar.

7. Its source.

8. Its chemical reactions and its preparation on a large scale.

In planning the investigations of these problems we were in the fortunate position of having the whole-hearted collaboration of several trained workers, not only in my own department (E. C. Noble, J. Hepburn, and J. K. Latchford), but also in that of internal medicine under the direction of Professor Duncan Graham (W. R. Campbell and A. A. Fletcher). We were also afforded, by the Connaught anti-toxin laboratories of the University under the direction of Professors J. G. Fitzgerald and R. J. Defries, the facilities necessary for the preparation of the extracts on a large scale, and through the generous support of the University and the Carnegie Corporation it was made possible later to add several others to the group of workers.

THE EFFECT OF INSULIN ON THE METABOLISM OF CARBOHYDRATES AND FATS IN EXPERIMENTAL PANCREATIC DIABETES.

From the time carbohydrates are absorbed, mainly as glucose, into the blood of the portal circulation until they are completely oxidized, changes in chemical structure are constantly occurring. These consist, partly, in a condensation of several glucose molecules to form glycogen, and partly in a splitting of the molecule, proceeding through various intermediate stages. The intermediary substances formed at each stage are doubtless in a certain state of equilibrium, one with another. Many of them do not accumulate sufficiently to be detectable by chemical means, being changed into the next stage almost as quickly as they are produced, and at the present time we are limited, in our attempts to follow the various steps in the process, to observations on the concentration of glucose in the blood, the amount of glycogen deposited in the tissues, and the type of combustion occurring in the organism as a whole. In diabetes marked and significant alterations occur in each of these; the blood sugar rises to a high level, glycogen practically disappears from the liver and becomes decreased in the muscles—except the heart, in which it increases—and the nature of the combustion process, as revealed by the behaviour of the respiratory quotient, becomes changed so as to indicate that no carbohydrate is being oxidized. It is safe to assume that the increase in blood sugar is secondary to the other changes, but it is difficult in the present state of knowledge to understand the relation. It has been a favourite hypothesis that the primary fault in diabetes is in the oxidation of glucose, or in the chemical changes which precede, and that this leads to the drafting to the tissues from the glycogen reserves of a plethora of sugar; a sort of forced-feeding process, as it were. Another hypothesis is that the glucose molecule must become altered in some way before it can be either oxidized or condensed into glycogen, and that this alteration does not occur in diabetes. Through the work of Irvine and his school much light has been thrown in recent years on the structure of the sugar molecule, and it has been shown that besides the well known α and β varieties of glucose, in which the oxygen linkage is between the first and fourth carbon atoms, there is also a third variety, called γ glucose, in which the oxygen ring is displaced from the normal stable position. The detection of the γ sugars depends mainly on a comparison of the reducing and polarizing powers of the solutions, and, by using this method, Winter and Smith have recently published results which they interpret as showing that the alteration in the glucose molecule alluded to above is the production of γ glucose. According to this view, γ glucose is a necessary preliminary stage in the oxidation of glucose, and, if we suppose that it is also so for its condensation into glycogen, the interrelation of the three changes found in diabetes becomes immediately intelligible.

I have dwelt on these theoretical considerations because of their great interest in connexion with the mechanism of the action of insulin. For it is a significant fact that this hormone, when administered to depancreated animals, completely restores to normal each of the three functions. We have repeatedly found, for example, that when it is

given along with carbohydrate to depancreated dogs, very large quantities of glycogen become deposited in the liver and that the respiratory quotient becomes raised in the same manner as it does in normal animals with carbohydrate alone. We know also from the work of Banting and Best on depancreated dogs that it reduces the blood sugar to the physiological level, that the glycosuria disappears, and that the well-being of the animal becomes normal in every regard.

We must conclude, therefore, that insulin is necessary for the metabolism of carbohydrate, and we may suppose that its function is in some way related to the preparation of the glucose molecule for combustion and condensation. But as to whether this preparation consists in a change of α , β glucose into the γ variety cannot as yet be considered as settled. Dr. G. S. Eadie¹ has repeated Winter and Smith's observations, and by strictly following their instructions has obtained similar results. Briefly stated, these results are that after removal of the proteins by phosphotungstic acid and alcohol from normal blood, the dextro-rotatory power of the filtrate is lower than it should be as calculated from the reducing power, and on standing twenty-four hours the differences become less, and later they practically disappear. On the other hand, when blood from diabetic patients or hyperglycaemic animals is used, the dextro-rotatory and reducing values correspond from the start, or the former may exceed the latter. Administration of insulin causes the diabetic blood to behave in these regards like normal.

The question is with regard to the interpretation of the results; as Hewlett has pointed out, this need not necessarily be that γ glucose is present, but rather that chemical changes have occurred in the very complicated procedure which is involved in the preparation of the solutions for the polariscope. Others have believed that the blood may contain, besides glucose, other carbohydrates or related substances having a relatively low dextro-rotatory power. Since this question is not yet settled I need not dwell on the further claim of Winter and Smith that insulin is capable of causing γ glucose to be formed when added to a solution of ordinary glucose along with an extract of liver. I may state, however, that we have so far been unable to confirm this result.

The action of insulin on the metabolism of fat in diabetic animals is as striking as on carbohydrates, and here again its effects are revealed by chemical changes occurring at both ends, as it were, of the metabolic chain. In a few days after pancreatectomy in the dog, the blood and the liver contain excessive quantities of fat, although the extreme degrees of lipaemia sometimes encountered in diabetic patients have not been observed. There is also a certain degree of acetonuria, although again this is much less pronounced than it is in diabetes in man. Evidently the migration of fat in the body is abnormal, and its final oxidation seriously interfered with. When insulin is given along with carbohydrates to these animals, the acetonuria very promptly disappears, the blood fat, apparently more slowly, returns to normal, and the liver in a few days contains no more fat than that of a normal, similarly fed animal. As the glycogen increases in this viscous the fat correspondingly declines. We cannot state at which stage in the metabolism of fats insulin exercises its corrective influence, but wherever this may be there can be little doubt that it acts indirectly because of its effect on carbohydrates. It has been said that fat burns in the fire of carbohydrates, and although this generalization may, in some respects, be misleading, it is, nevertheless, a useful guide in interpreting the results with insulin.

THE THERAPEUTIC EFFECTS OF INSULIN IN DIABETES MELLITUS.

As would be expected, the effects of insulin on patients suffering from diabetes mellitus, in so far as these have been studied, are of the same general nature as those observed on diabetic (depancreated) dogs. Until the laboratory investigations had furnished perfectly definite results no serious attempts were made to carry the investigations into the clinic, nor would it have been permissible to do so in view of the failures of previous workers to provide an extract that did not cause toxic symptoms.

Without animal experimentation we would be no further to-day in the treatment of diabetes, and in the undoubted saving of human life which has been the result, than we were a little over a year ago when the treatment was first applied on any considerable scale.

In studying the results obtained by administration of insulin to patients, we must bear in mind that two factors may come into play that are not involved in the experiments on completely depancreated animals. One of these is dependent on the fact that there probably still remains in the majority of diabetic patients a certain remnant of pancreatic tissue capable of secreting insulin. The other is dependent on certain differences between laboratory animals and man, related in part to peculiarities in the type of metabolism and in part to the greater development of the nervous system, to which the frequent occurrence of coma as a serious symptom of the clinical form of the disease is due. Moreover, we must remember that subjective phenomena can be studied only in man.

The first case in which a complete study of the therapeutic value of insulin was made was that of a boy fourteen years of age, exhibiting all the classical symptoms of the disease; treatment by dietetic control had resulted in no improvement. Daily injections of insulin between January 23rd and February 4th, 1922, reduced the blood sugar, even to the normal level, greatly diminished the glycosuria, and often caused it to disappear, removed entirely the ketonuria for a period of some days, and, perhaps most striking of all, "the boy became brighter, more active, looked better, and said he felt stronger." There could be no doubt as to the great therapeutic value of the treatment when judged both by the improvement in the objective and subjective symptoms of the disease. This, along with other cases treated by insulin, and reported by Banting, Best, Collip, Campbell, and Fletcher, is only the first of a large number, exhibiting the disease in all its forms, that have been most carefully studied in numerous clinics both in Canada and the United States and in Great Britain, and I cannot refrain from expressing my admiration for the excellent spirit of collaboration which has made this possible. I am not qualified, nor even if I were would it be in place for me here, to discuss further the clinical results, but I may say that there can be no doubt that insulin is capable, when wisely used under controlled conditions, of saving many cases of diabetes which would otherwise inevitably succumb to coma, or to one of the many other complications of the disease. It is also certain that as an adjunct to dietetic control, treatment with insulin greatly improves the nutritive condition and increases the resistance towards infections, thereby prolonging the expectation of life. The subjective condition and the sense of well-being of the patients are also vastly improved. As to whether continued treatment with insulin will enable the diabetic patient to recover any of his lost power of producing this internal secretion in his own pancreas, nothing definite can be said at present. It is at least significant in this connexion that the islet tissue develops considerable powers of regeneration after much of it has been destroyed as a result of ligation of the secreting ducts of the pancreas, as has been shown by R. R. Bensley to happen, at least in the rabbit and guinea-pig. Arising as it does in these cases from outgrowths of the duct epithelium, may it not be possible in man, in whom the pancreas has not been completely destroyed by disease, that new islet tissue will become regenerated when, by administration of insulin, the strain to produce this hormone endogenously is removed?

THE EFFECT OF INSULIN ON THE BLOOD SUGAR OF NORMAL ANIMALS.

Returning now to the more purely physiological aspects of the subject, let me briefly lay before you what is known regarding the effects of insulin on normal animals. In the consideration of these results we must bear in mind that the conditions in the normal animal are fundamentally different from those obtaining in the diabetic. In the normal animal there is probably available at all times a sufficient amount of insulin for every purpose for which it is required, so that the addition of more from without

upsets the metabolic balance and thereby induces an abnormal state. In the diabetic, on the other hand, the injection of insulin only restores to the body a hitherto missing part of the metabolic machinery and enables it to run smoothly again.

Within a few minutes of the intravenous or subcutaneous injection of a moderate dose of insulin, the percentage of sugar in the blood begins to fall, and it continues to fall, more or less in a straight line, for a period which varies from about half an hour to several hours, according to the animal; afterwards the curve may gradually become less steep or it may begin to rise again.

The prompt onset and the steep nature of the fall in blood sugar are characteristic features of the action of insulin, and are quite unlike the hypoglycaemic effects that may be produced by administration of other substances, such as phosphorus or the insulin-like materials that have been prepared by Collip and others from clams and from various vegetable sources. Indeed, the character of initial fall is such as to give the impression that the process responsible for it must be one operating within the blood itself—an intravascular glycolysis. But this cannot be the case, since we have been unable to find that the addition of insulin to defibrinated blood kept under sterile conditions at body temperature outside the body has any influence on the rate at which sugar disappears; neither is there any difference in this regard between the blood of normal animals and that of animals injected with insulin at varying periods preceding the withdrawal of the blood. Evidently, therefore, the locus of action of insulin is extravascular; it is in the tissue cells. We must conclude that the insulin sets up some process by which, as it were, a vacuum for sugar becomes established in these cells, so that sugar is removed from the blood. One may imagine that in the tissues there exists a certain balance between the concentration of free glucose, the complex carbohydrates from which it may be derived, and the various derivatives into which it breaks down prior to its oxidation. In other words, one may speak of a certain tension of glucose as being necessary in the tissues and consider the action of insulin as having the effect of reducing this tension so that glucose diffuses into them from the blood in order to maintain it.

After about half an hour the blood sugar in the rabbit usually begins to rise again, or at least the fall to become much less pronounced, indicating that the effect of insulin is becoming neutralized by the liberation of sufficient glucose from the glycogen stores of the muscles and liver. This, of course, means that the hypoglycaemia following insulin will be much more prolonged and will ultimately become much more pronounced in animals provided with small reserves of glycogen than in those that are rich in glycogen. Such can readily be shown to be the case, and it is significant that a well fed animal may withstand many times the dose of insulin that would prove fatal to one that was starved.

It is customary to imagine that the glycogenic mechanism of the liver is so finely attuned to respond to the concentration of sugar in the blood that glycogenolysis immediately sets in when this falls below the normal level. That such is not the case following insulin may depend on the fact that the glycogen, which is first of all called upon to maintain the glucose tension in the tissues, is that of the muscles themselves; but I will not here venture further into the various interesting consequences of such an hypothesis. It is at least significant that the glycogen of the muscles, as well as of the liver, is usually reduced in animals that have been given large doses of insulin (McCormack and O'Brien and Dale and Dudley). On account of its practical significance it should be pointed out that it is because of the poverty of the glycogen reserves that the blood sugar of diabetic patients treated with insulin is more apt to fall suddenly to a dangerously low level than in normal persons.

THE HYPOLYCAEMIC SYMPTOMS.

When the percentage of blood sugar has fallen to about 0.045, curious symptoms supervene. They were first observed in rabbits, and in a typical case consist of a violent convulsive seizure in which the animal throws itself over sideways, usually first in one direction, then in the other,

with the head retracted and the hind limbs in an extended position.

The condition is not unlike that caused by strychnine or by acute asphyxia, except that certain groups of muscles are less affected by the convulsions than others. After a period, usually of from 30 to 60 seconds, the convulsions cease and the animal lies on its side in an unconscious state, with rapid, shallow breathing, and perhaps showing running-like movements of the extremities. The exact condition of the animal at this stage may, however, vary considerably; well fed and therefore glycogen-rich animals often sit in apparently normal fashion between the convulsive seizures, which are often brought on by attempts to move. After a varying period the comatose stage is followed by another convulsive seizure, and these phases may continue alternately for an hour or more, the convulsions becoming feebler and feebler and the rectal temperature falling, until at last the animal dies of respiratory failure. After death rigor mortis sets in at once. The arterial blood is venous in colour and clots very quickly. Preceding the onset of typical convulsions there are usually premonitory symptoms of hyperexcitability and evidence of hunger; sometimes, however, paralysis of the extremities is the first symptom.

The symptoms vary somewhat in other animals. In the dog the first signs are usually very rapid breathing, restlessness, and general hypersensitivity; muscular twitching then becomes evident and the sphincters may relax. Barking is often a prominent symptom, and there may be frothing at the mouth. At this stage, or as the first symptom, convulsions not unlike those seen in the rabbit may supervene, and between them the animal lies on its side, showing violent twitching of the musculature, almost a tetany. It is evidently unconscious. The rates of breathing and of the pulse increase; inspiration is usually short and jerky, and inspiratory tetanus not infrequent, so that artificial respiration may have to be applied. Attempts to get on its feet often cause convulsive seizures, and, during recovery, the muscles of the extremities, particularly the anterior, are seen to have entirely lost their power of co-ordinate action. In etherized dogs even massive doses of insulin have no immediate effect on the blood pressure.

In the cat the symptoms are like those in the dog, profuse salivation, mewing, and relaxation of the sphincters being especially marked.

In the mouse kept at room temperature, A. Krogh observed that insulin may only cause the animal to become weak, but its temperature falls to a very low level. If it be kept in an incubator at about 28° C., however, characteristic symptoms supervene—convulsions and coma, preceded by paralysis of the legs—with doses of insulin, which, when compared on a body-weight basis, are only about one-fifth that necessary for rabbits.

This influence of body temperature is particularly interesting, since it probably explains the apparent immunity of frogs even to massive doses of insulin. We failed to note any symptoms even in three days after injecting insulin, but A. Krogh has since found that they occur in a day or so later—a fact which we have confirmed.

In man, in whom the subjective symptoms can be interpreted and cardio-vascular changes more accurately observed, the picture is different. When the blood sugar reaches about 0.075 per cent. the patient experiences extreme hunger and a sense of fatigue. He usually becomes anxious and may lose his emotional control. Actual tremor of the musculature is rarely seen, but there is a definite sense of tremulousness and some inco-ordination for fine movements. "Vasomotor phenomena are common; pallor or flushing, sometimes one after the other; a sense of heat, of chilliness, almost always a profuse sweat" (Banting, Campbell, and Fletcher). At lower levels of blood sugar, acute mental distress, mental disturbances, delirium, and finally coma, with loss of the deep reflexes, supervene.

The correspondence between the onset of definite objective symptoms and a blood sugar percentage of 0.045 in laboratory animals is remarkable. Sometimes, as in starved animals receiving a large dose of insulin, the blood sugar may be found to be considerably below this level when convulsions first appear. Very occasionally it is decidedly higher, and immediately after a convulsion the sugar may rise somewhat. These facts suggest that the symptoms are the result of the lowering of the tension of glucose in the tissue cells below a certain critical level—a condition of "glucatonia," we may call it—to which the hypoglycaemia runs parallel. This view is supported by the striking manner in which the symptoms are almost instantly removed by administration of glucose, and it is of great significance that other hexose monosaccharides have only a slight beneficial effect, although of course their injection causes a marked rise in the reducing power of the blood. Mannose and laevulose seem to be somewhat more efficient than galactose. None of the pentoses or disaccharides has any effect. Injection of epinephrin, subcutaneously, also removes the symptoms, no doubt because

it excites the production of glucose out of the glycogen reserves. It is of use, therefore, only when these reserves are considerable, and should not be depended upon to antidote the symptoms of glucatonia in diabetic patients, because in them the glycogen stores are apt to be low. A similar relation between the percentage of blood sugar and convulsive symptoms had previously been described by Mann and Magath in dogs deprived of the liver; administration of glucose also removed the symptoms, other monosaccharides having only a slight effect.

There can be little doubt, then, that the symptoms are related to a lowering of the glucose tension in the tissues. But we cannot imagine that this, in itself, is responsible for the violent stimulation of the respiratory and muscular centres that evidently occurs. Some unphysiological condition must have become developed as a result of the glucatonia, and it has been suggested that anoxaemia may be an important factor. Olmsted and Logan point out that there is a remarkable resemblance between an insulin convulsion and an asphyxial convulsion, and that the arterial blood is invariably intensely venous in colour when convulsions supervene. "It may possibly be that through the lowering of blood sugar certain oxidative processes become depressed to such a degree that the brain cells are affected in much the same manner as in asphyxia." Whatever may be the nature of the toxic condition, it is clearly on the cells of the pons and medulla that it acts. It does not, like strychnine, act on the motor centres of the spinal cord, since insulin causes no convulsive symptoms in a decapitate cat, although it may lower the blood sugar to well below the convulsive level (Olmsted and Logan).

On what particular detail in the chemical structure of the molecule the remarkable specificity of glucose in antidoting the symptoms may depend cannot as yet be said, but it is evident that, by studying the relative antidoting value of various sugars and substituted sugars, we are furnished with an interesting method for determining the biological significance of the different side-chains and of the other molecular arrangements of the glucose molecule.

THE PHARMACOLOGICAL ASSAY OF INSULIN.

The extent to which insulin lowers the blood sugar forms the basis for its pharmacological assay, one unit being originally defined by us as the amount which is required to lower it to 0.045 per cent. within four hours in a rabbit weighing two kilograms and after twenty-four hours' fasting.

Since convulsions supervene at this level, their occurrence can be used as a check on the assay, and when small animals, such as mice, are used, so that large numbers can be injected with varying amounts of insulin, it can be made by observing the onset of the symptoms alone. In this case one mouse unit is defined by A. Krogh as the quantity of insulin which can cause convulsions in 50 per cent. of the animals within two hours, precautions being taken to maintain the body temperature and to standardize the diet. Dale and Burn have also tried this method of assay.

Even when rabbits are carefully selected so as to correspond in weight and in every other particular, are similarly fed prior to the preliminary starvation period, and are injected intravenously so as to obviate any uncertainties in rate of absorption, the assays, as judged either by the blood sugar curve or the occurrence of convulsions, do not always correspond. Differences in glycogen content, which can never be accurately adjusted by dieting, are probably largely responsible. We have attempted to circumvent these difficulties by confining the observations on the blood sugar to the first half-hour after the insulin injections, hoping in this way to circumvent the influence of the glycogen reserves, but with no conspicuous success. Probably the most accurate method of assay is that worked out in Toronto by Dr. F. N. Allen on depancreatized dogs. The principle of this method, which was suggested by our clinical associates, is to determine the number of grams of glucose that a given quantity of insulin can cause to be metabolized while the animal is on a diet containing enough carbohydrate so that there is some glycosuria. He has found that the carbohydrate equivalent of insulin, as it is called, varies somewhat with the weight of the animal and the carbohydrate balance, but that it is satisfactorily constant when these conditions are made uniform. It is not likely that this method will succeed in the clinic, because of the varying degree to which different diabetic patients retain the power to produce insulin.

I have said enough about this problem of assay to indicate how difficult it is. It is probably advisable to point out, however, that it has been found advantageous to adopt, for clinical purposes, temporarily at least, a unit

which is one-third that of the standard unit as defined above. This is done to obviate the necessity of prescribing fractions of a unit for the treatment of mild cases of diabetes.

THE EFFECT OF INSULIN IN EXPERIMENTAL HYPERGLYCAEMIA.

Insulin also lowers the blood sugar in every known form of experimental hyperglycaemia, whether this be produced by the addition of glucose exogenously or endogenously. With regard to the former type, Eadie has found that when insulin is injected into rabbits coincidentally with, or at varying periods preceding, the injection of sugar the curve of the resulting hyperglycaemia becomes greatly altered. When the insulin injection coincides with that of sugar the curve rises to the same height as with sugar alone, but it returns much sooner to the normal level, and when the sugar is given some time after the insulin the curve is not only of shorter duration but is also much less in height. The maximal influence of insulin in suppressing the curve is when the sugar is given at an interval of about 75 to 90 minutes after the insulin. At longer intervals the curves progressively rise again, the suppressing influence of insulin having, as a rule, disappeared in about eight hours. We have attempted to use these facts as the basis for a method of assay, but with no great success.

Of the endogenous forms of experimental hyperglycaemia, we have studied the effect of insulin on those due to piqure, to epinephrin, to asphyxial conditions, and to anaesthetics, and Stewart and Rogoff have studied it on the peculiar form due to morphine. The hyperglycaemia which becomes rapidly developed in glycogen-rich rabbits in all these conditions is greatly diminished, if not entirely absent, in animals injected with insulin, this depending, obviously, on the amount injected. Most attention has been paid to epinephrin hyperglycaemia, because in this case alone is it possible to control the intensity of the hyperglycaemia by varying the dosage. This, it will be seen, offers a method for the assay of insulin, and Eadie has found that when equal quantities of epinephrin are injected into a series of uniformly fed rabbits along with varying quantities of insulin, there is usually a definite mathematical relation between the dose of insulin and the extent to which the blood sugar rises.

Further interest attaches to the antagonistic action between insulin and epinephrin, with regard to the blood sugar, because they are both hormones, although it is questionable whether the hormonic action of epinephrin is of importance to the animal. It is significant in this connexion that Stewart and Rogoff have found that the hypoglycaemic effect of insulin is not perceptibly different in adrenalectomized than in normal animals, thus showing that the adrenals are in no way linked with the internal secretion of the pancreas in the control of sugar metabolism.

The observation that insulin can prevent the hyperglycaemia due to ether is of clinical importance, because it is probably on account of this hyperglycaemia that a large part of the risk of surgical operations on diabetic patients is due. It should be pointed out, however, that the hypoglycaemic action of insulin is much more readily demonstrated by having the animal well under the effects of insulin before the ether is administered, than by giving insulin to animals that are already anaesthetized. This would seem to indicate that diabetic patients should be treated with insulin before being anaesthetized, and, indeed, that this preliminary treatment should extend over several days so that the reserves of glycogen may be augmented. I am informed by my clinical colleagues that insulin is of little avail in combating post-operative coma if its administration is delayed until after the operation.

An interesting antagonism between pituitrin and insulin has been described by Burn. Given in large doses, pituitrin, like insulin, may inhibit the hyperglycaemia due to epinephrin or to ether, but when insulin and pituitrin are given together to normal animals, instead of there being a greater fall in blood sugar than insulin alone would produce, there

is little change, or, it may be, a rise. A dose of pituitrin which in itself has little effect on blood sugar is thus capable of neutralizing the hypoglycaemic action of insulin. This observation has been confirmed by Logan and Olmsted, who have also found that it is necessary to give enormous amounts of insulin to cause any decided decrease in the hyperglycaemia which is present in decerebrate cats still retaining the pituitary gland. When this gland is absent, on the other hand, not only is the hyperglycaemia much less pronounced, as Bazett had suggested it would be, but insulin promptly lowers the blood sugar and hyperglycaemic convulsions can readily be induced.

All of the forms of experimental hyperglycaemia just alluded to are primarily due to a rapid breakdown of the glycogen of the liver, and that produced by epinephrin may therefore be taken as a type. This does not, of course, warrant the assumption that some have made, that a hypersecretion of this hormone is the underlying cause of the hyperglycaemia in the other forms. Indeed, Stewart and Rogoff have conclusively shown that such is not the case, with the possible exception of that caused by morphine. Taking epinephrin hyperglycaemia as a type, then, it seemed of interest to compare the amount of glycogen left in the livers of uniformly well fed rabbits after they had been given equal doses of epinephrin, some of them at the same time also receiving insulin. This observation, made by E. C. Noble, has shown very definitely that the insulin protects the glycogen against breakdown. In one experiment, lasting eight hours, the livers of two animals receiving epinephrin alone contained 1.40 and 1.96 per cent. of glycogen, whereas those of three others which were given insulin along with the same amounts of epinephrin contained 8.12, 6.4, and 3.64 per cent. of glycogen. It is of interest to note that it was necessary to give about forty times the usual dose of insulin to cause convulsions under these conditions, and that sometimes the convulsions occurred while there was still over 10 per cent. of glycogen remaining in the liver. The results show quite clearly that insulin antidotes the glycogenolytic action of epinephrin.

THE PHYSIOLOGICAL MECHANISM BY WHICH INSULIN LOWERS THE BLOOD SUGAR.

We are now arrived at a stage where it will be advantageous to consider briefly the possible mechanism of the hypoglycaemic action of insulin. We have presented evidence that this is not because it stimulates increased glycolysis within the blood itself, and have concluded that insulin in some way lowers the tension of glucose in the tissue cells.

Further evidence that insulin causes the sugar to pass from the circulating fluid into the tissues more quickly than normal has been furnished by perfusion experiments on the isolated mammalian heart.

Hepburn and Latchford, using Locke's method, found, as previous workers have, that an average of 0.9 mg. of glucose per gram of (rabbit) heart per hour disappears from the perfusion fluid. By adding insulin to the perfusion fluid the average sugar consumption rose to over 3.0 mg., and in some cases reached the value of over 4.0 mg., the highest without insulin being below 2 mg. They did not find that insulin affected the heart beat, nor could they detect any significant differences in the glycogen content of the hearts used in the two groups of experiments.

Hoping to be able to demonstrate a similar effect of insulin on the sugar content of fluid perfused through skeletal muscles, these workers attempted to perfuse the hind limbs of various laboratory mammals, but, although every possible precaution was taken to avoid interruption of the circulation prior to starting the perfusion, or a fall in temperature, they could never succeed in preventing either marked oedema or vaso-constriction. This failure led them to compare the sugar concentration of blood removed at the same time from the femoral artery and vein, and from the portal vein, of anaesthetized or decerebrated animals, and to see whether insulin would cause the differences normally existing between these bloods to become changed. It was found not to do so. McCormack has since found that this method is also incapable of revealing an increased retention of glucose as a result of insulin, either in the muscles or in the liver of depancreated animals, in which, as we have seen, insulin certainly causes glycogen to be formed. This shows that the method is not sufficiently sensitive for the purpose for which it was employed.

The evidence of the observations on glycolysis and the perfused heart leave little doubt, however, that the glucose is lost in the tissue cells, and the problem narrows itself down to the cause for its disappearance. The possibilities

that may be considered are: its polymerization into glycogen, its oxidation, and its reduction to substances similar to fatty acids.

With regard to its possible conversion into glycogen, the fact that insulin causes large amounts of this polysaccharide to become deposited in the liver of depancreated animals when carbohydrates are given would seem at first sight to lend support. But the conditions, as already pointed out, are fundamentally different in the normal organism. As a matter of fact, it has been shown that insulin given to normal rabbits, instead of causing glycogen to be deposited, has the opposite effect, and either prevents the deposition of glycogen when it is given along with carbohydrates to previously starved animals (McCormack and O'Brien) or causes the stores of this substance to become reduced when given to well fed animals (Dale and Dudley).

Further evidence that insulin does not stimulate formation of glycogen in normal animals has been obtained by Noble by perfusion experiments on the liver of the turtle. When each of the two lobes is perfused for several hours with Ringer's solution containing glucose, under exactly similar conditions except that the fluid on one side contains insulin, the relative amounts of glycogen in the two lobes were found to be the same as they usually are. Since the normal variation may be considerable, the method is not a very delicate one, and on this account Noble has also used that of observing the sugar content of the perfusion fluid, as recommended by Snyder, Martin, and Levin. By comparing this for equal periods of time, with and without the addition of insulin, no differences could be detected. The only objection to the experiment is that insulin may not act quickly enough in the case of cold-blooded animals to make it possible in the time of perfusion for measurable quantities of glycogen to be deposited.

Clearly, therefore, none of the disappearance of sugar from the blood of normal animals which follows the injection of insulin can be accounted for by its polymerization to glycogen. Why, then, does insulin cause glycogen to be deposited in the diabetic animal, and why does it inhibit the breakdown of glycogen in experimental hyperglycaemia? The reply, based on the considerations already set forth, is that an excess of insulin, beyond that actually required for the control of carbohydrate metabolism, develops towards glycogen an action which is the reverse of that which it has under normal conditions. When insulin is given to the diabetic animal, on the other hand, it supplies a previously missing agency necessary for the oxidation and polymerization of glucose. To explain the retarding effect which insulin has on glycogen breakdown in experimental hyperglycaemia, we may suppose that the sugar which is produced by this process is a mixture of active and inactive forms, and that insulin, by tending to produce the former in relative excess, disturbs the balance existing between the sugars and glycogen, so that less glycogen is broken down. This suggestion assumes some sort of balanced action between glycogen and its hydrolytic products, for which, however, there is no experimental evidence.

The possibility that glucose disappears because excess of insulin causes its oxidation to be increased has been investigated by observing the respiratory exchange.

In the experiments of Dixon, Eadie, and Pember, dogs and rabbits were used. In the former, the results show that insulin causes a very marked increase in oxygen consumption and in respiratory volume and rate, but very little change in the respiratory quotient. These changes do not, however, begin to be evident until from 30 to 60 minutes after giving the insulin, by which time the blood sugar has completed its initial fall and stands at about 0.060 per cent., which indicates that the sudden call for sugar on the part of the tissue cells cannot be attributed to its increased oxidative breakdown. There can be little doubt that the increased metabolism is dependent upon the hyperexcitability and the increased muscular tone of the animal. It reaches its acme when convulsions supervene, and it develops either steadily or intermittently. Evidently, as the tension of glucose declines, conditions become established within the nerve centres which excite them and which may be dependent upon an intracellular lack of oxygen. That the development of the symptoms depends on the lowering of the glucose tension is supported by the observation that their severity is lessened by the injection of glucose.

In rabbits, in which it will be recalled increased muscular tone is not so marked a symptom as in dogs, the increase in oxygen consumption does not set in until the convulsions occur—indeed, there is often, if not usually, a drop both in this and in respiratory volume. Sometimes in rabbits the respiratory quotient rises decidedly. It would be rash at this stage to draw definite conclusions from these results, but it is quite plain that the initial fall in blood sugar, which is primarily what we must attempt to explain, is not due to any increased combustion of glucose.

The remaining possibility that glucose is reduced to substances related to the fatty acids receives some support in certain of the observations from the behaviour of the respiratory quotient. This may rise to over unity without

there being any sufficient degree of hyperpnoea, and of consequent "washing out" of CO_2 from the blood to account for it. To be quite certain of this latter possibility, Dixon and Pember have examined the blood removed from dogs at regular, frequent intervals after giving insulin without finding that there is any significant change in the CO_2 -combining power. This observation, by the way, also shows that there can be no production of acid substances as a result of insulin, a fact which has also been demonstrated by F. N. Allen from observations on the behaviour of the acid excretion by way of the urine.

We are, therefore, at a loss to explain the cause for the initial disappearance of sugar from the blood; it is not converted into glycogen, its oxidation in the tissues is not augmented, at least until after increased muscular activity sets in, and if some of it does become reduced to fat-like substances this is not an invariable process.

THE SOURCE OF INSULIN IN THE HIGHER ANIMALS.

The evidence that insulin is derived in the higher animals from the islets of Langerhans and not from the acinar (zymogenous) cells of the pancreas has, until recently, been of an indirect nature.

Continuing the work of Diamare and Laguesse on the comparative anatomy of the islets of Langerhans, Rennie, in 1905, described in detail their relationship to that of the zymogenous tissue in numerous Teleostei. He found, particularly in *Lophius piscatorius*, *Myoxocephalus*, and the *Pleuronectidae*, that the islet cells exist as separate encapsulated glands lying in the mesentery quite distinct and apart from the bulk of the zymogenous tissue, which is usually spread as narrow bands along the branches of the mesenteric blood vessels. These glands he called Principal Islets, and he found the largest of them to be situated near the anterior pole of the spleen.

Although the more recently discovered methods of differential staining for islet cells have not been successfully applied to the principal islets, no one has doubted the conclusion of Diamare and Rennie that they are homologous with those of the islets of Langerhans of the mammalian pancreas. The absence of these structures in the zymogenous tissue in the Teleostei supports this view, as does also the fact that in the Elasmobranchi, in which there are no principal islets, the pancreas itself, which is a compact gland, contains abundant islet tissue. In collaboration with Fraser, Rennie investigated the effect of the principal islets, administered by mouth, on diabetic patients, but without results that could be considered to show definite amelioration of the symptoms. In one case an extract of the gland was injected subcutaneously, but the development of toxic symptoms made a repetition of the experiment inadvisable. In view of the now well established fact that insulin is quite inactive when given by mouth, it is clear why these earlier observations should have failed to yield satisfactory results. Recently, we have reinvestigated the problem, and have found very definitely that alcoholic extracts of the principal islets cause a profound lowering of the blood sugar of normal and diabetic animals, including man, when they are injected subcutaneously or intravenously, whereas similarly prepared extracts of the zymogenous tissue have no such effect—indeed, not infrequently, rather cause the percentage of blood sugar to increase. In contrast to these results, an extract of the pancreas of the skate, which contains islet tissue, has a marked insulin effect,* and so also had one prepared from the intercaecal fat of the trout (in which the islets and zymogenous tissue lie separately embedded). The islets are, therefore, the only source of insulin in the bony fishes, and we may conclude that they are also so in the mammals, since in them none of this hormone is produced after pancreatectomy.

This conclusion is supported by the experiments on duct ligation and on grafting already referred to, and by the experiments of Banting and Best, although in all of these there still remained the possibility that partially degenerated or regenerated zymogenous tissue might be the source of the hormone.

Much attention has been given in recent years to the mechanism of control of the secretion of glands producing internal secretions, and, since many of those concerned in the production of external secretions are under nerve control, it has been supposed that this must also be the case with the ductless glands. It cannot be said, however, that much success has attended the various endeavours to demonstrate this nerve control. Since the islets of Langerhans are provided with a rich nerve supply, which is believed to come in large part from the right vagus, and since an increased discharge of their internal secretion, insulin, would be revealed by the behaviour of the blood sugar, McCormack and O'Brien have investigated the effect which is produced on the latter by electrical stimulation of the right vagus, in decerebrate cats and in etherized dogs and rabbits. To avoid cardio-inhibitory

effects, the nerve has either been stimulated below the origin of the cardiac fibres, or it has been allowed to undergo partial degeneration. The fact that hyperglycaemia follows stimulation of the great splanchnic nerve further suggests the possibility that hypoglycaemia might result from stimulation of the vagus.

Similar observations were made some years ago by de Corral, working in Asher's laboratory, with results which are at least suggestive. Those of the present investigation are more definite, although not entirely convincing. In many cases no change can be detected in the level of the blood sugar following the stimulation, but in several others there is a prompt fall, which is usually of a temporary nature, in spite of continued stimulation. In one or two cases, however, stimulation with slow, weak induced shocks for a long period has caused a gradual decline in blood sugar, in one case to 0.070 per cent., with a return towards the normal level after removal of the stimulus. It is of great interest in connexion with these experiments that Best and Scott have recently discovered that insulin can be prepared from blood by the usual methods.

THE CHEMICAL REACTIONS AND THE PREPARATION OF INSULIN.

The method of preparation developed by Collip depends on the solubility of insulin in concentrations of ethyl alcohol up to about 90 per cent., above which it becomes precipitated.

Since most proteins are insoluble at concentrations of alcohol that are considerably below this, they can be removed from the original extract of pancreas (which is made with 50 to 60 per cent. alcohol) by fractional precipitation after the extract has been concentrated by evaporation *in vacuo*. A watery solution of the precipitate which settles out at a concentration of 90 per cent. alcohol is insulin. It can be evaporated to dryness *in vacuo* without loss of potency, and the powder contains about 14 per cent. of nitrogen, is free from phosphorus (Doisy, Somogyi, and Shaffer), but contains sulphur. A solution gives a distinct biuret test, but only faintly those of Millon and Hopkins-Cole. The insulin is readily absorbed by kaolin and charcoal, and considerable losses are apt to occur in passing it through a porcelain filter. It does not dialyse through membranes or collodion sacs; it is precipitated by half-saturation with ammonium sulphate, by barium salts of weak acids, and by picric acid. The solubility in water and alcohol is determined by the H-ion concentration, the iso-electric point being at a pH between 5 and 6. Watery solutions at a pH below 5 can be boiled for several hours without any loss in potency, but this occurs more rapidly when the pH is about 7. The potency quickly vanishes in the presence of pepsin or trypsin. In acid solution insulin is relatively stable to mild oxidizing and reducing agents. Insulin is also soluble in phenol and the cresols, glacial acetic acid, and formamide. It is insoluble in most other organic solvents (Malony).

These reactions, and its destruction by pepsin and trypsin, suggest that insulin is of the nature of an albumose, but it is possible that it is really only adherent to this protein, and is itself of much simpler chemical structure. This view is supported by the fact that we have been unable on several occasions to detect any biuret reaction with insulin prepared from the pancreas of the skate or to obtain from this source precipitates containing it by means of high concentrations of alcohol, or by adjustment of the iso-electric point. Whether it is really a protein or not, it is significant that all the methods now used for its purification are based on reactions that are characteristic of colloids: precipitation of the iso-electric point (Doisy, Somogyi, and Shaffer), or by picric acid followed by the regeneration from the picrate as a hydrochloride (Dudley), or by benzoic acid (Malony) or by half-saturation with ammonium sulphate.

It is highly creditable to those who have been responsible for producing insulin on a large scale for clinical use that they should have succeeded in so short a time in their work.

It may be assumed that insulin is present in all animals, and since structures similar to the islets of Langerhans have not been described in the invertebrates, it is likely that in them its site of production is delegated to other structures. What these may be in the more highly developed invertebrates is as yet undetermined, but it is of interest that Collip, shortly after isolating insulin from mammalian pancreas, prepared it from clams, and suggested that it must be present wherever glycogen is found. Later he prepared a substance like it from yeast, thus confirming

* These extracts have an unusually marked effect in causing violent convulsions.

Winter and Smith. The insulin prepared from these sources appears to differ from that prepared from pancreas, in that its action in lowering the blood sugar is delayed, sometimes for a day or so, which is also the case with an insulin-like substance prepared by Collip from various vegetable sources, and styled by him, in recognition of these differences, "glucokinin." Best and Scott have also succeeded in preparing insulin, or insulin-like substances, from various other sources. It would appear, therefore, that the distribution of hormones capable of accelerating the disappearance of glucose from the blood is widespread, not only in the animal kingdom but in the vegetable as well; it is probable, however, that these do not all have the same physiological action. In the investigation of this we must bear in mind that many substances have been known for long to be capable of lowering the blood sugar—for example, peptone, hydrazin, certain mineral salts, phloridzin, etc. All of these also cause glycogen to disappear from the liver, and indeed certain of them cause structural changes in this organ. Although it would certainly be desirable to have substances like glucokinin having a more prolonged action than insulin in depressing the blood sugar, we must exercise some caution in recommending them for clinical use until it is certain that they have no deleterious effects on the liver. It is possible also that these substances merely stimulate the secretion of insulin from the pancreas of the animal into which they are injected, or serve as precursors for its production. If such be the case, then they can have little value in diabetes.

I have endeavoured in this review to adhere strictly to ascertained facts, and have refrained from venturing to speculate with regard to the numerous problems in metabolism, both of animals and plants, for the investigation of which insulin may prove a useful instrument. If I have succeeded in giving a bird's-eye view, as it were, of the results that have been obtained, I shall consider that my efforts have not been in vain, and I shall feel repaid if, as an outcome, others join us in the work on insulin.

REFERENCE.

¹ G. S. Eadie: BRITISH MEDICAL JOURNAL, July 14th, 1923, p. 60.

THE RELATION OF FAULTY NUTRITION TO THE DEVELOPMENT OF THE EPITHELIOMA CONTAGIOSUM OF FOWLS.

(With Special Plate.)

BY

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EPITHELIOMA CONTAGIOSUM (avian variola; fowl-pox; chicken-pox; epitheliosis; pigeon-pox; canker) is a disease of birds which has been described as follows:

It is characterized by "the appearance of wart-like nodules, varying in size to that of a pea, or slightly larger, on those parts of the skin of the head where feathers are few or absent, such as the nostrils, openings of the ears, the comb, wattles, and the eyelids, the last named being one of the most common sites. The nodules are encapsuled, firm in consistence, with a caseous centre, and usually remain discrete, but a number situated closely together form a mulberry-like mass. In some cases the contents resemble fatty tissue, except for being more vascular; in others they are warty and contain flakes of coagulated material very much like small clots of milk. Lesions are also found on the buccal mucous membrane, the affected parts of which become covered with thick whitish-yellow exudate, in appearance resembling that of ciphtheritic false membrane."¹

"Marx and Sticker, in 1902, demonstrated that the disease was due to a virus which was capable of passing through a Berkefeld filter, and their results were confirmed by Juliusberg and Hertzog. Burnet, however, obtained very varying results in his filtration experiments. He found that the virus was frequently arrested by the Berkefeld filter, although the infective material had been largely diluted with water. It has not been possible to cultivate the virus artificially, and its nature remains a matter for further investigation. It exists in the blood, internal organs, and in the fluids expressed from the lesions, and is possessed of considerable

resistance to the action of heat* and antiseptics. The disease can be transmitted by rubbing juice from the lesions into an area of skin from which the feathers have been removed, or which has been lightly scarified, and the lesions then produced are confined to the part inoculated. According to Burnet, it can also be transmitted by intravenous inoculation and by feeding, and the lesions develop at the predilection seats of the disease."¹

"The microscopic examination of the epithelioma of the skin shows, according to Polowinkin, the epithelial layers thickened and the epithelial papillae elongated inward; in the papillary layer the vessels are dilated and, like those of the subcutis, surrounded by migrating leucocytes; in addition there are small haemorrhages into the tissue of the papillary layer. Cell-inclusions are found in the swollen epithelium, similar to Guarnieri's pox-bodies (Loewenthal, Burnet). In a general way the characteristic features of the efflorescences are composed of a more or less pronounced hyperplasia and proliferation of the cell elements of the skin, which sooner or later succumb to degeneration. This is associated with the extension of the process to the external skin and there follows a condition which, according to Reischauer, is similar to true pox; the difference lies mainly in the fact that in bird-pox the more chronic character of the disease causes the nodular and tumour formation to predominate."²

My purpose here is to record the occurrence of epithelioma contagiosum in pigeons under conditions which indicate that nutritional factors may play an important part in its development. The observations have another and a more general interest, inasmuch as they tend to show that a certain state of faulty nutrition, and of deranged metabolism, favours the entry into the body and the activity of an invisible virus possessing the specific property of inducing epithelial new growth.

Experiment.

Polyneuritis columbarum was produced in a number of pigeons by exclusive feeding on washed milled rice. Twelve of them were "cured" at the height of the disease by artificial feeding with green *mung dal*. Within forty-eight hours of the disappearance of polyneuritic symptoms they were placed in an open-air pen with sixty healthy stock birds, and had access to the mixture of grains found to be most suitable, in this climate and at this altitude, as a stock food for pigeons. It consists of one part of green *mung dal*, one part of *ragi*, and four parts of *cholum*. Thus, the birds, after the cure of the polyneuritis, received a food containing a sufficiency of vitamins and other essentials for their maintenance in perfect health. Owing to their poorer nutrition when put into the stock-pen, they were less able to protect themselves against attack by their more lusty companions. The lessened ability to escape injury and to recover from it is an important effect of faulty food on the animal organism, and in this instance abrasions about the head—the most frequent seat of injury—appear to have favoured the entry of the virus of epithelioma contagiosum. In general, then, the twelve "cured" birds were more subject to injury than their fellows. Nevertheless, as weeks passed they appeared to prosper; their plumage improved and their body weight rose. Six weeks to three months after they were put into the stock-pen seven out of the twelve developed epithelioma contagiosum (Fig. 1), while their sixty healthy well fed companions remained free from it.

The growths occurred about the angles of the mouth, on the eyelid, the nostril, in the neighbourhood of the ear, and in the floor of the mouth beneath the buccal mucous membrane (Fig. 1). Their histological characters are shown in the photomicrographs (Figs. 3 to 6). The epithelial downgrowths stimulated in appearance those seen in such a condition as carcinoma of the penis in man. The epithelial new growth was infiltrative to a limited extent, had local destructive properties, and its cells tended to undergo rapid degeneration.

The nodules were emulsified, and the emulsion was rubbed into lightly scarified surfaces at the angle of the mouth of six healthy and well-fed pigeons. In two of these diphtheritic false membranes developed, and one of them died from the extension of the false membrane backwards, resulting in interference with swallowing and in involvement of the air passages; the second recovered. The other four were unaffected. In none did the inoculation of the emulsified nodules cause infiltrative new growth of epithelium. It would seem, therefore, that these well nourished birds were resistant to the influence of the growth-inducing virus, since those affected by its inoculation exhibited only superficial lesions. It appears likely that the virulence of the virus was not great; it was great enough to cause typical lesions in birds whose nutrition was faulty, but not so great as to be capable of causing infiltrative lesions in healthy, well-nourished birds.

*In this respect resembling the growth-promoting substance evolved during the process of autolysis of tissue cells.²