Section of Therapeutics and Pharmacology

President—DOROTHY C. HARE, C.B.E., M.D.

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Protamine Insulinate

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FORMERLY, diabetics were half-starved invalids, unable to withstand the stress of ordinary life and easy victims to common infections, very often with disastrous results. The introduction of insulin into therapy has meant a complete revolution of their fate. Experience has more than fulfilled the hopes we had fifteen years ago, and the success was so great that at first nobody thought of the possibility of improving this remedy. But the demands upon it have been increasing, because we are not satisfied unless our patients—even our most severe cases—are able to work and enjoy life to the same extent as normal individuals.

The ordinary method of injecting insulin a fraction of an hour before a meal is very suitable in milder cases where the pancreas is still able to deliver sufficient insulin for the post-absorptive period, although unable to meet the greater demand during food absorption. The injected insulin then deals with the alimentary hyperglycæmia.

Where the disease has developed further, the ability to produce insulin naturally is still more reduced, even to the extent of being insufficient for the control of the post-absorptive state. It is only in such severe cases that the ordinary method does not bring about absolute success in so far as considerable oscillations in the blood-sugar are noticeable.

The most pronounced cases demand very careful and frequent examination, a balanced diet, and several daily insulin injections to prevent hyperglycæmia, glycosuria and acidosis on one hand, and hypoglycæmia on the other. The practicable number of daily injections is limited, and hence we must often tolerate very considerable fluctuations in the blood-sugar. This fact makes it desirable to try to improve our technique.

The difficulties mentioned can be considerably reduced or completely abolished by a suitable diet, including an appropriate distribution of the food, especially the carbohydrates, among the various meals, bearing in mind the rate of glucose absorption from different kinds of food. These considerations, necessary in any difficult case, are very important, but they do not fall within the scope of our present subject, which deals only with the development of methods for a better timing of the insulin effect through modifications of the insulin preparations themselves. The earlier insulin preparations were not highly purified, and although more or less unsatisfactory in other respects, did have perhaps some advantage over the pure products in being more slowly absorbed.

This raised the problem of artificially retarding absorption which has been more or less in the mind of everybody working on diabetes. It was mentioned publicly as early as 1923 at the Scandinavian Congress for Internal Medicine at Oslo, and perhaps even earlier.

Our Institute has been very much interested in this problem ever since, and we have tried many methods without satisfactory results. Three different ways have been tried to obtain a retardation of the insulin effect :—

(1) The emulsion of watery insulin solution in oil or suspension of dry insulin in oil. This method has been successful in a number of cases, as can be seen from the very fine curves in Dr. Otto Leyton's monograph (1933), but does not seem applicable in every case.

(2) Injection of a vasoconstrictor substance, e.g. adrenalin, together with insulin, as exemplified in the very extensive use of vasoconstrictors along with local anæsthetics (Clausen, 1934).

(3) The third method—which at the present moment seems to be of greatest promise—depends upon the fact that the insulin is injected as a more or less insoluble compound.

The dose of soluble preparation can be augmented by increasing the concentration of the solution or the amount of the solution injected. In both cases the rate of absorption will also be increased, either by the greater concentration or by the increase of the surface through which the absorption takes place. The increase of the surface with increasing volume depends upon the shape of the depot, and we may say that the increase of surface with increasing volume is very considerable because the shape of the depot is very far from the ideal sphere, as can be seen from the X-ray picture (fig. 1) taken two minutes after injecting 1 c.c. of a suitable iodine compound.

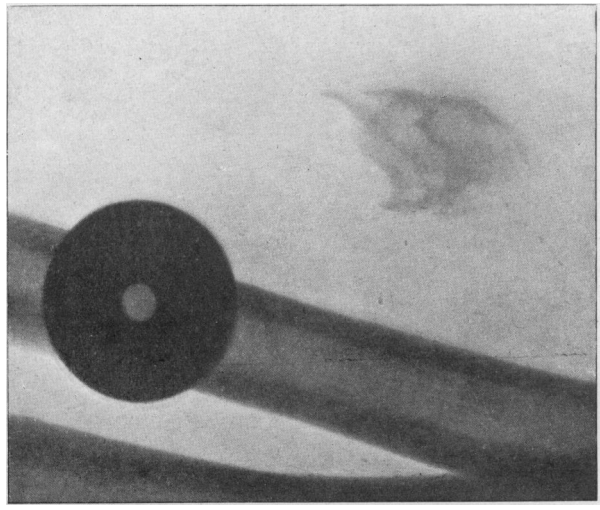

FIG. 1.—To the left a coin about the size of a florin ; to the right the depot.

The insoluble compound, in contrast to the soluble, allows a variation in the dose without varying either the volume or the concentration of insulin in the liquid phase. Theoretically an injection of a suspension of an insoluble compound will give a constant rate of absorption until all solid particles are dissolved.

One of the first—if not *the* first—reports of experiments with such an insoluble compound, was made by Leyton in his earlier quoted monograph, and dealt with the use of phosphotungstate. Our Institute has limited the experiments to such compounds as could be expected to be either destroyed or rapidly excreted from the body, feeling that the continued injection of substances not fulfilling these requirements might perhaps sooner or later give rise to unpleasant sequelæ. In restricting

the experiments to such substances as are destroyed or rapidly excreted, such sequelæ are not with certainty prevented, but they are much less likely.

In a preliminary communication (1936) we have given a brief summary of earlier experiments, omitting a certain amount of unsuccessful work. (Among other unpublished experiments were some using tannic-acid compounds of insulin. They were abandoned because of local reactions. I mention them now because reports of work along that line have recently been published elsewhere (Bischoff 1936)). In this preliminary communication we reported solubility experiments with some compounds of protamine and insulin,[1] and the results obtained through the use of such compounds in the treatment of diabetes. As soon as it was realized that some success could be expected through the use of such compounds in so far as they were non-toxic, in doses far greater than those required during treatment, and that a greatly prolonged effect of the insulin could be easily demonstrated clinically, several questions arose. First of all, what kind of protamine would be most suitable ? It is obvious from the preliminary communication that there seem to be differences between various kinds of protamine compounds, and that the solubility in serum is different for different protamines.

Accordingly we could not expect to have obtained one of the most suitable protamines at the very beginning, and so we began collecting ripe sperm from different fishes in the Mediterranean, Atlantic, and North Sea, as well as in the Danube, Elbe, Vistula, and the Scandinavian rivers. It seems certain that the group of protamines which according to Kossel (1928) are called monoprotamines, and which contain as basic group only the amino-acid arginin, are more suitable than the di- or tri-protamines. The investigation of the material is still in progress, but it appeared that a closer investigation of one of these substances was necessary to determine the conditions under which the experiments had to be carried out. For this work we have chosen a protamine, as far as we know not hitherto prepared, which seems to us more suitable than the others, and which has the further advantage of being easily prepared from easily obtainable raw material, protamine of the rainbow trout, *Salmo irideus*. The protamine obtained was called salmiridin. Much of our chemical work has concerned the quantitative relations of insulin and salmiridin, which we have felt must be cleared up before further progress could be made in examining other protamines and before the use of different proportions of protamine to insulin was tested in the clinic.

The purpose of protamine compounds of insulin was to alter the iso-electric point from the pH 5 of ordinary insulin to the iso-electric point of the tissue fluid somewhat over 7. As soon as this was effected and the compound was found successful, the desirability of combining in some way the ordinary insulin with protamine insulin immediately raised the question of a method for determining the quantitative proportion between protamine and insulin, because any surplus of protamine would precipitate more or less of the ordinary insulin added.

This led to a physico-chemical investigation of the nature of such compounds, which has been carried out by Mr. Krayenbühl. The results will be published in full elsewhere. Even if such researches are outside common medical interest, I feel I ought to mention them here, because some of our results may influence the choice of preparation to be used in the clinic.

The line we have followed in investigating the quantitative proportions between insulin and protamine has been the precipitation of a known amount of insulin with a known amount of protamine, with the subsequent separation of the resulting sediment from the supernatant fluid, to which thereafter small amounts of protamine or insulin, respectively, are added. If free insulin is present in the fluid, the addition of protamine will cause the formation of a precipitate invisible to the naked eye,

[1] We have termed these compounds insulinates to indicate that they are of related nature to salts. Criticism has, however, been raised against the term, and we have no objection against the simple term—protamine insulin.

but measurable by a nephelometer. By thus measuring the surplus of insulin and protamine, respectively, it could be easily shown that there exists a definite proportion of protamine to insulin, dependent on the pH, but within wide limits independent of the concentration, and characterized by the proportion of protamine to insulin being the same in solution and in precipitate. For this proportion the term " isophane " is suggested (meaning " same appearance ", i.e. irrespective of whether a surplus of protamine or insulin is added). All other proportions of protamine to insulin will contain a surplus of insulin or protamine, which can be removed by washing the precipitate and thus appears to be present in a looser combination.

During experiments with insulin crystals some irregularities in the isophane proportion were observed. Thanks to the remarkable work of Scott (1934) on the influence of zinc upon the formation of crystals, it could be easily demonstrated that the irregularities depended upon the content of zinc, and that under certain circumstances zinc could replace part of the protamine in the combination.

Protamine does not have any influence when injected together with insulin in solution. It has to be precipitated at a suitable pH before being injected. This means a certain practical disadvantage ; on the other hand it allows of the biological estimation of the insulin without—or at any rate with but slight—disturbance from protamine.

The stability of the insulin solution decreases with increasing pH. However, precipitated protamine insulin is stable—even under ordinary circumstances—for many weeks, and in cold-storage for months. The addition of zinc perhaps further stabilizes the product. For our own work, and to satisfy the demands from parties interested in diabetic research, we have had to prepare protamine insulin on a larger scale. It soon appeared that the patients were being discharged on that treatment, and so we had to make a preparation which was constant from batch to batch. For this purpose we have selected an isophane compound with only a very small surplus of protamine to make sure that the isophane point is reached. The isophane compounds have not the maximum prolongation effect, but on the other hand can be mixed with insulin to make heterophane compounds with surplus of insulin.

Before going into the matter of the clinical test I would like to say a few words on the use of zinc, and I wish to express my thanks to Professor Best for having informed me at an early date of the advantage of zinc protamine insulin.

It is more or less generally accepted that the dose of zinc required is so small that it can be taken as absolutely harmless. This of course is all right if it is completely absorbed. If not, there is a slight possibility—but still a possibility—of local trouble ; and that is the reason why I personally reserve the zinc for those cases in which protamine alone is not satisfactory.

A subcutaneous injection of protamine into laboratory animals and normal human subjects is tolerated without any discomfort or noticeable effect, in far greater doses than those used in treatment with protamine insulin. Continued injections are well tolerated by animals. From our knowledge of the constitution of the protamines we may expect that their split products are the same as the split products of the proteins in food. Still the possibility exists that the continued use might give rise to unforeseen trouble, e.g. locally. Consequently we preferred to treat a few cases for more than a year before we started a longer series of experiments.

These few cases have guided us as to the management of other cases, and at the same time we have been guarded against any sudden appearance of unexpected difficulties in a large number of cases.

The next point is to demonstrate unequivocally the special effect of the new remedy and to investigate how this special quality, if any, can be used in treatment. The endless chain of remedies for diabetes and the many discussions on treatment tell a sad story of how the capricious course of the disease, with its tendency to rapid spontaneous changes, together with the optimism of the patients and the doctors,

have obscured the facts and led to false conclusions. The statement that some patients do well under a new treatment—and better than before — may be very encouraging for them and their doctors, and for the rest of us very interesting and even inspiring, but it is very far from being clinical evidence; such evidence can only be obtained if the whole clinical treatment is arranged as a scientific experiment, keeping all other conditions constant and adequately controlled, so that the real experimentum crucis is obtained.

We are much indebted to our collaborator, Dr. Niels B. Krarup, who has undertaken to carry out such experiments in our hospital, and under controlled conditions has made experiments on diabetics, making careful observations of blood-sugar, urine-sugar, and acidosis (1935).

Fig. 2 clearly demonstrates the prolonged effect, and this experience has been confirmed by others, especially British and American observers.

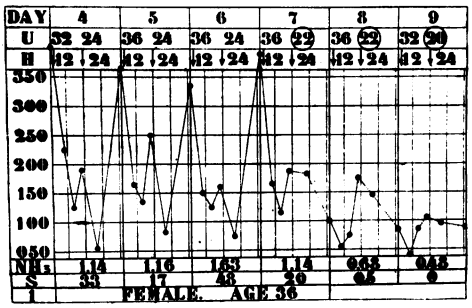

FIG. 2.—First line: number of the day of treatment. Second line: number of units injected. One ring round the figure means that protamine insulin was administered. Third line: hours of the day. The ordinates represent sugar in blood in mgm. per 100 c.c. Under the graph the excretion of ammonia and sugar in g. per day.

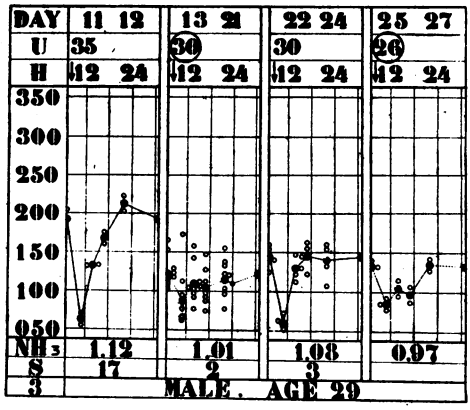

FIG. 3.—The black dots represent average figures; the open circles, individual observations. This figure and some of the following figures, have been copied from *Krarup* (1935).

Fig. 3 exemplifies experiments in which the protamine has been given in one daily dose. Everybody who is convinced of the harmlessness of the protamine will agree that protamine insulin is indicated as soon as one daily dose of ordinary insulin does not keep the blood-sugar within reasonable limits. There are cases in which one daily dose of protamine insulin can keep the blood-sugar within the same limits as two doses of ordinary insulin.

Protamine insulin is very well suited for an evening dose (figs. 4 and 5, p. 16) in cases in which the blood-sugar rises considerably during the latter part of the night, especially when this rise is accompanied by acidosis, in some cases amounting to a condition very near to precoma. Ordinary insulin is used in such cases during the day to meet the special requirements during and after meals, but during the night the slower effect of protamine insulin keeps the blood-sugar far better under control than does ordinary insulin.

Often the blood-sugar may remain low for up to fourteen hours, but it should be remembered that the effect stops very abruptly, causing a sudden rise in the blood-sugar.

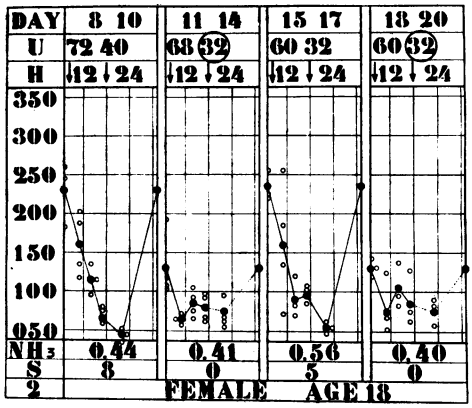

FIG. 4.

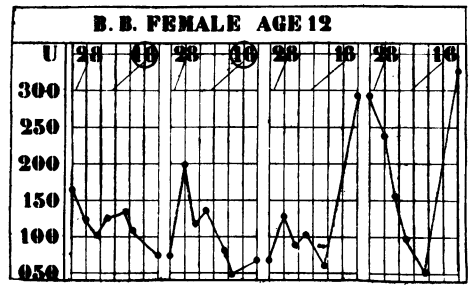

FIG. 5.

Occasionally, some time may pass before the full effect of the protamine insulin appears (fig. 6). If the morning dose is not reduced, slight hypoglycæmic symptoms may occur (fig. 7).

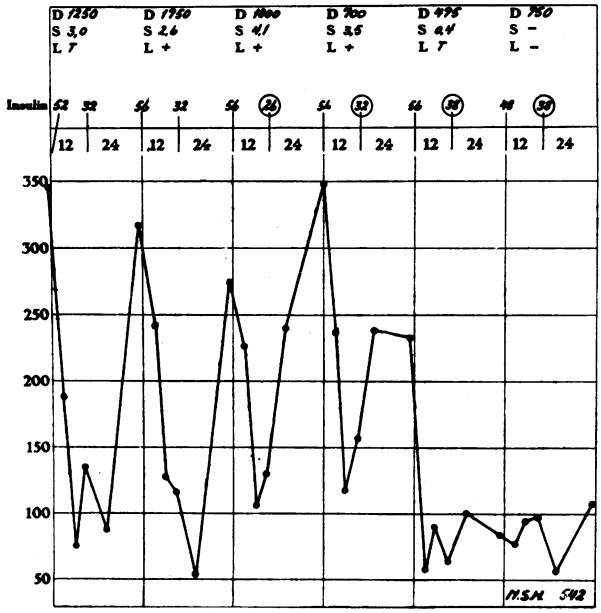

FIG. 6.—*D*, diuresis; *S*, sugar; *L*, Legal's test.

In cases in which the effect is not sufficiently prolonged, the evening injection must be postponed, usually one or two hours, to ensure a normal fasting blood-sugar; or a preparation that works still slower must be employed, for instance zinc protamine insulin.

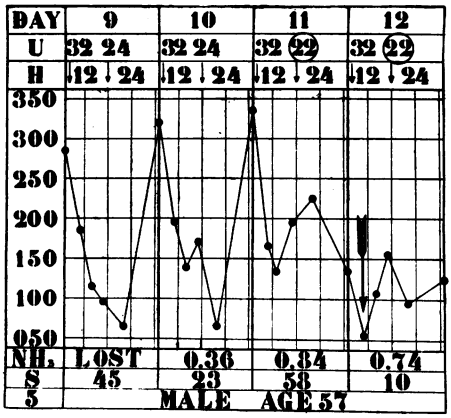

FIG. 7.—The arrow indicates slight hypoglycæmic symptoms.

In some cases, especially in children, two daily injections of protamine insulin have proved useful (figs. 8 and 9).

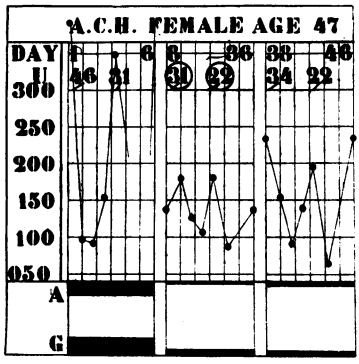

FIG. 8.—*A*, acidosis; *G*, glycosuria.

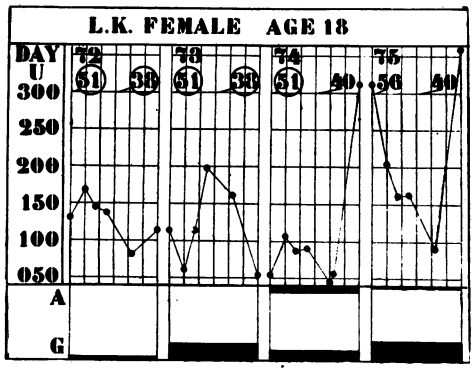

FIG. 9.

Very often we would like to have a combination of the effect of the ordinary and that of the protamine insulin, viz. cases in which ordinary insulin causes a sudden fall in the blood-sugar and protamine insulin is not able to prevent a very high alimentary rise. Such an effect can be obtained, in some cases with great success by injecting at the same time, but in different places, ordinary insulin and protamine insulin. It is also possible to use a heterophane compound with a surplus of insulin. This has given fairly good results (fig. 10), but it is still uncertain whether the duration of the effect of such compounds is quite as long as that of protamine insulin.

Further, it has to be decided whether the standard preparation should be used for all cases, or matters so arranged that the individual patient may be able to draw into his syringe from two different bottles a mixture most suitable for his individual case.

Hitherto I have spoken only of the laboratory findings; they are objective. Patients, however, have also stated that they feel better and are less tired, especially when formerly they might have had extreme blood-sugar values, either high or low.

The slower development of the hypoglycæmic symptoms gives them ample warning so that they can take precautions in time. It is interesting, however, that slight hypoglycæmic symptoms tend to remain for a rather long time and resemble the physiological state experienced by numerous normal individuals when exercising during fasting or hunger. Many patients tend to increase the quantity of food and insulin without experiencing any trouble. Good results have been observed in complicated cases.

Surgical cases have become easier to deal with. In three cases complicated with Graves' disease, gratifying results, first on the diabetes and then on Graves' disease, were obtained. One of them afterwards was operated on successfully, while the improvement was so great in the two others that we did *not* insist upon operation. Children have generally done very well under protamine treatment; one case, a girl, aged 4, after being treated for months in expert hands with three daily injections of ordinary insulin, was sent to our hospital in the early days of protamine treatment. Six weeks of protamine insulin treatment was sufficient to improve the child's appearance out of recognition. Sometimes the general condition is so much improved that I prefer not to speak about the treatment but advise you to try it for yourselves.

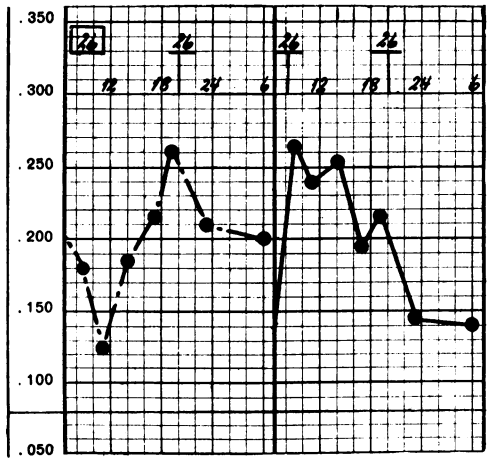

FIG. 10.—The underlined figure = units of ordinary protamine insulin. The figure in the square = units of heterophane compound with surplus of insulin.

Many patients, both children and adults, who earlier were repeatedly admitted to hospital either hypoglycæmic or semi-comatose, now keep out of hospital. We have had only one case admitted on account of hypoglycæmia following a large overdose taken during alcoholic intoxication.

It appears that the dose of protamine insulin can be reduced to the extent of 15% compared with ordinary insulin.

But we must not forget the other side. It is not in every case that we succeed in keeping the blood-sugar within reasonable limits, and at the present stage we are not able to say with certainty in which cases this can be done. As a result of hitherto unexplained individual differences—most likely in the resorption from the subcutaneous tissue—some cases are found which do not react to the protamine insulin in the typical way. There is also a group of patients in whom the wide fluctuations in the blood-sugar are caused by changes in their need of insulin and neither an optimal

dose of ordinary nor of protamine insulin can be fixed, but even in such cases the protamine insulin has some advantage, because the patients have better warning against the hypoglycæmic attacks.

REFERENCES

BISCHOFF, F. (1936), *Am. J. Physiol.*, **116**, 239-244.
CLAUSEN, V. (1934), "Kliniske Undersøgelser over Insulinresorptionens Paavirkelighed af Adrenalin, Pituitrin og Ephetonin," Dissertation, Levin and Munksgaard, Copenhagen (Danish.)
HAGEDORN, H. C., NORMAN JENSEN, B., KRARUP, N. B., and WODSTRUP, I. (1936), *J.A.M.A.*, **106**, 177-180.
KOSSEL, A. (1928), "The protamines and histones", London.
KRARUP, N. B. (1935), "Clinical investigations into the action of protamine insulinate," Dissertation, G. E. C. Gad, Copenhagen.
LEYTON, O. (1933), "Diagnosis and treatment of diabetes mellitus", London.
SCOTT, D. A. (1934), *Biochem. J.*, **28**, 1592-1602.

Discussion.—Dr. OTTO LEYTON: Dr. Hagedorn confers another boon upon diabetics by supplying a compound of insulin which in many cases converts a life of hazard to that of comparative monotony.

Everyone who has had any wide experience of protamine insulin has been able to confirm the results obtained by Dr. Hagedorn and his co-workers, but it must be remembered that its action is not the same in all patients, and it is possible that chance may arrange matters so that the first dozen cases upon which it is tried derive no benefit. My own experience to begin with was of that kind, but further trials showed that in over 50% of the cases the prolonged action of protamine insulin prevented not only severe hypoglycæmic attacks, but also the rise of the concentration of sugar in the blood in the early morning.

Time does not permit my giving details of cases in which, in spite of much juggling with food, four injections of insulin hydrochloride were required daily and even on four injections a day slight hypoglycæmia occurred, but upon two injections of protamine insulin daily the sugar content of the blood was maintained between 0·08% and 0·15%. I, like many others, have placed patients upon a single dose of protamine insulin first thing on awakening, and observed whether that would maintain the sugar content of the blood between the limits mentioned above throughout the twenty-four hours. Occasionally this has been astonishingly successful, even when a single dose of more than 40 units has been required. Naturally the distribution of carbohydrate had to be adjusted. Usually about 35% was given at breakfast, about two hours after the injection, and 50% at luncheon, 10% at tea-time, and 5% at the evening meal. The total carbohydrate was often well over 200 grm. In some cases, however, this form of treatment was disastrous, hypoglycæmia developing about 11 a.m., or the insulin being apparently quite ineffective.

It is always wise to inform patients who would like to try a single dose of protamine insulin in exchange for two doses of insulin hydrochloride that it is experimental.

I have found protamine insulin invaluable in those patients who are developing remissions. It has in the past been difficult to decide how to treat patients who at one time, perhaps, have needed more than 60 units of insulin daily and have gradually improved, so that 12 units daily suffice. If the 12 units are given as a single dose in the morning, frequently the sugar content of the blood rises above 0·18% after the evening meal. Protamine insulin will often control the sugar content in these patients throughout the twenty-four hours, and as time passes the dose may, in favourable cases, be diminished, and finally discarded.

The variable action of protamine insulin is occupying the attention of many. It seemed likely that the site of injection might modify the duration of its action. A few observations were made contrasting the effect of injection into a vein, into a muscle, into adipose tissue, and under the skin. The differences were not sufficiently great to account for the variability.

It would be of interest to see, and, provided that it has not already been tried, we shall observe at the London Hospital the effect of subcutaneous injection of insulin phosphotungstate and other insoluble salts of insulin upon those patients who develop hypoglycæmia under treatment with protamine insulin, in order to see whether they possess a special power of dissolving protamine insulin or whether some other explanation must be sought. Although it might not be safe to inject a salt of phosphotungstate acid for a long period, it is unlikely that harm would follow a dozen injections.

At the present time, when I am asked about protamine insulin, my advice is as follows: If the sugar content of the patient's blood is well controlled upon two doses of insulin hydrochloride daily and the dose required exceeds 20 units a day—leave well alone, unless there be some special difficulty due to some disability of the patient which prevents him from injecting himself with insulin, and it is impossible to arrange for an evening injection. If, on the other hand, the sugar-content of the blood cannot be kept below 0·15% throughout the twenty-four hours without hypoglycæmia developing in spite of subsidiary meals, then try the effect of protamine insulin, and there is slightly more than an even chance that it will prove successful.

Dr. GEORGE GRAHAM said that through Dr. Hagedorn's courtesy, he had been given some of the protamine insulin in order to try its effect on patients. The new preparation was of great value for those whose blood-sugar fluctuated from say, 300 mgm. per 100 c.c. at 8 a.m. to 50 mgm. per 100 c.c. at 12 noon, and back to 300 mgm. per 100 c.c. at 5 p.m. These patients required a third dose of insulin at midday and a fourth also at midnight, and this procedure was often very inconvenient. The protamine insulin, with its slower action, had the effect of keeping the blood-sugar at a lower level. In one severe case the dose of insulin required had been reduced by 60 units. Dr. Leyton had spoken of the absence of overdoses with the new insulin. Patients who did not recognize the signs of overdose with ordinary insulin recognized those due to the protamine insulin more easily. This was not always the case, and two of his patients had had very severe overdoses even in the day-time as well as in the night. He agreed that it would be a great advantage to have insulin of quadruple strength, as a dose of 100 units of double strength had a large volume, and was painful for patients. He asked Dr. Hagedorn whether it would be possible to have an insulin which would act less quickly than the ordinary insulin but more quickly than the present protamine insulin. The choice of the right kind of insulin for any individual case would become a difficult one but this must be faced, because the patients already presented these difficulties, and it was desirable that a solution should be found.

Dr. IZOD BENNETT said that he could fully endorse the tributes to the utility of protamine insulinate in the treatment of diabetes in children. With Dr. Morton Gill he had published the excellent results obtained in a series of children under treatment at his special clinic at the Middlesex Hospital; in every case it had been possible to reduce the total dose and to diminish the frequency and severity of reactions, and all the children felt better on the new product. He thought that physicians tended to ignore the dread with which many diabetic patients regarded reactions.

In adult cases he had had many favourable experiences with the new product, but he also encountered variations in its actions from day to day, which sometimes made it difficult to control the glycosuria. He agreed with Dr. Graham that the introduction of these new products was going to render still more difficult the task of those treating diabetes, for it would seem that a number of substances, each with a different action, would soon be available, and each would present special problems with regard both to dosage and to the dietetic side.

He hoped that the British manufacturers would not regard the situation with too much complacency, but would endeavour to meet the problem of supplying reliable products on the lines of these new insulins which had undoubtedly come to stay. The profession owed a great debt to Dr. Hagedorn for his work.

Mr. F. H. CARR said he wished to assure Dr. Izod Bennett that the British manufacturers of insulin were not neglecting the manufacture of protamine insulin. Realizing that great confusion would have resulted from the issue of protamine insulin in different forms, the British manufacturers had decided to act in unison in this matter, and in this the Ministry of Health was giving them every encouragement. Dr. Hagedorn had been most helpful in placing at the disposal of the companies making "A B" insulin all the necessary information to enable them to make protamine insulin.

Dr. H. C. HAGEDORN (in reply) said that a preparation at the strength of 80 units had been tried, but it was still doubtful whether it had exactly the same effect as a double dose of 40 units strength. As it was obvious that the use of protamine insulinate might lead to unexpected happenings, there was every reason to proceed with caution.