after the injection of pentnucleotide 10 c.cm. This amount was given twice daily, and when the patient was distressed coramine was injected. On July 24th there was again a blotchy eruption on the abdomen and lower part of the chest, and some exudate was seen on the posterior pharyngeal wall. The tongue and mouth were very dry and there was some diarrhoea. On the next day dysphagia was marked and the pulse became intermittent. On July 26th a small anal ulcer was seen. The throat sloughs were now very large, covering most of the fauces as before. On July 28th Cheyne-Stokes breathing was noticed when the patient was asleep. Much offensive sputum was coughed up, suggesting that sloughs were separating further down the pharynx and oesophagus. The report on the sputum was:

"Direct examination shows epithelial cells, no pus, many Gram-positive and Gram-negative cocci. Cultures resulted in growths of the following organisms, the approximate percentage of each being shown from a rough colony count: haemolytic *Streptococcus longus*, 10 per cent.; pneumococcus, 40 per cent.; *Micrococcus catarrhalis*, 50 per cent."

A second anal ulcer was observed on July 28th. By July 29th the patient was becoming increasingly weaker, the sclerotics appeared yellow, the sputum was foul-smelling and yellow in colour, and by 2 a.m. on July 30th she was semi-comatose; by 7 a.m. she was completely unconscious, and died at 1.30 p.m. The temperature rose to 106.6° F. before death.

Examination of a blood film on July 29th showed that:

"Many of the lymphocytes are larger than normal, with more cytoplasm, which stains more deeply than usual."

Immediately after death the patient became a vivid yellow colour, with purple patches beginning in the face and continuing down the limbs and all over the body.

Comment

In this patient for some reason the manufacture of polymorphs became irregular early in 1934, and the attacks in April and November must, in view of subsequent events, be regarded as due to a condition of mild agranulocytosis. The red cells appeared to be unaffected, and a count on December 24th revealed 4,650,000 red cells per cubic millimetre, with haemoglobin 74 per cent. Polymorphonuclear leucocytes were known to be absent for four days in the penultimate attack. The pentnucleotide treatment appeared to be responsible for the recovery in December, 1934, but in the illness of July, 1935, the patient never showed any response to it, the total white count continuing to fall till it reached the very low figure of 210 per cubic millimetre. Death in coma with hyperpyrexia also occurred in the case reported by Fairley and Scott.³

It would seem that this malady is more prevalent than is at present thought to be the case; it might therefore be wise in all cases of severe tonsillitis to examine the blood in order to exclude the possibility of agranulocytic angina.

Table of Blood Counts

		Percentage of Polymorphs in Differential Count	Total White Cell Counts
December	22nd, 1934	0.0	705
"	24th, "	0.9	580
"	26th, "	19.0	1,720
"	30th, "	41.0	3,070
January	5th, 1935	44.1	3,715
"	18th, "	19.4	2,030
"	23rd, "	24.8	3,750
"	28th, "	43.0	3,500
"	30th, "	44.0	2,670
February	5th, "	36.0	4,120
"	12th, "	42.0	3,850
"	19th, "	50.5	3,500
March	15th, "	59.2	7,080
June	20th, "	44.5	3,060
July	22nd, "	0.7	2,200
"	24th, "	0.0	930
"	26th, "	0.0	560
"	27th, "	0.0	270
"	29th, "	0.0	210

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SOME EXPERIMENTS WITH PROTAMINE INSULINATE

BY

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AND

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One of the most obvious difficulties in the use of insulin in diabetes is its short-lived and often too violent action after injection. Thus in many severe diabetics a high fasting blood sugar (300 to 400 mg. per 100 c.cm.) may be rapidly reduced to normal, perhaps to hypoglycaemic levels, in three to five hours, but is maintained at this level for only an hour or two and, when the insulin action is over, rises to hyperglycaemic levels as quickly as it fell, with a return at the same time of glycosuria and ketonuria. Such patients run the risk of a dangerous alternation between clinical hypoglycaemia and hyperglycaemia with ketosis. Attempts have been made to slow and prolong the action of insulin, but the first important success has only recently been obtained by Dr. Hagedorn and his colleagues in Denmark. After two to three years of work they have discovered a compound of insulin and a protamine (protamine insulin*) which by virtue of its low solubility in the tissue fluids is very slowly absorbed. One of us had the privilege of seeing the clinical results obtained by the Danish workers, and their experiences,^{1,2} with confirmatory results from America,³ have just been published.

The advantages claimed are: (1) Its prolonged action, which can therefore keep the blood sugar normal for longer than ordinary insulin. It is stated to act "about twice as long," and a normal fasting blood sugar can therefore be obtained before breakfast. (2) A reduced liability to hypoglycaemia because of its slower and less violent action. The disadvantages pointed out in their publication are: (1) The instability of the protamine compound if kept for more than three to four weeks. (2) Its inability to control the hyperglycaemia which follows carbohydrate meals.

Hagedorn's present clinical practice is therefore to give insulin in the morning to deal with the day's carbohydrate food, and protamine insulin (P.I.) at night to keep the blood sugar in control until the next morning's dose. In our opinion some patients might find the use of two sorts of insulin very confusing.

By the courtesy of Dr. Hagedorn we have received a supply of protamine insulin for experimental purposes, and have obtained complete confirmation of his results. We have extended our experiments to investigate in more detail its clinical action and possible uses and limitations. In all our investigations the blood-sugar estimations were controlled by simultaneous urine samples tested for sugar and ketones by qualitative methods. The latter, which are seldom published, were in every instance confirmatory.

* "P.I." will be used for the new insulin compound and "I." for the present form.

Length of Action

The duration of action of P.I. is vaguely stated to be "twice as long" as that of ordinary insulin. It is important to know more exactly how long it can act, with a view to the possibility of controlling diabetes by one injection a day. This was tested on two young severe diabetics who showed rapid oscillations of blood sugar after ordinary insulin. The criterion of the duration of P.I. action was the time during which it could prevent a rise in blood sugar from endogenous sources alone. Therefore no carbohydrate food was given during these experiments.

ILLUSTRATIVE CASES

Table I shows results in a girl of 11 with severe diabetes whose blood sugar was normal four hours after 30 to 40 units of I., but always rose to 300 to 500 mg. eight to ten hours

TABLE I

Time	Hours After Injection	Mg. of Blood Sugar per 100 c.cm.				
		120	120	130	316	216
7 p.m.	0					
11 p.m.	4	50	111	—	308	208
8.30 a.m.	13½	352	51	148	325	221
10 a.m.	15		53		388	
2 p.m.	19		59			
3 p.m.	20		76			
4 p.m.	21		115			
5 p.m.	22		158			
Amount injected, 7 p.m.	...	32 units I.	36 units P.I.	16 units P.I.	16 units P.I.	12 units P.I.

after I. Different insulins and doses were given at 7 p.m. with 50 grams of carbohydrate, and no more food until the blood sugar rose. Thirty-six units of P.I. kept the blood sugar low, indeed at a slightly hypoglycaemic level for twenty-two hours, when a gradual rise of blood sugar with a simultaneous return of glycosuria and ketonuria terminated the experiment. A much smaller dose—16 to 12 units of P.I.—had variable effects, depending on the initial level at 7 p.m., but did seem to prevent the rise in blood sugar for about twelve hours.

TABLE II

Hours After 120 Units of P.I.	Blood Mg. of Sugar per 100 c.cm.	Urine		Food
		Sugar	Ketones	
24	69	0	0	
27	114	0	0	P. & F.
29	118	0	0	
31	104	0	0	P. & F.
33	114	0	0	
35	101	0	0	
37	79	0	+	P. & F.
39	95	0	+++	
41	67	0	+++	
48	54	0	++++	
51	68	0	+++	P. & F.
53	76	0	+++	
55	—	0	++	
55½	104	0	+	60 grams carbohydrate
57	279	+++	+	
59	200	+++	+	

P. & F. = Protein and fat meal.

In the second case there was a much longer action of P.I. after a large single dose of 120 units. The details of this experiment, showing interesting fluctuations in glycaemia and ketonuria, are given in Table II. This case, one of severe acute untreated diabetes in a girl of 22, was given an almost normal carbohydrate diet (250 grams) and one large dose—80, and then 120 units—of P.I. a day to see if one dose could control the diabetic condition. Within a week ketonuria was entirely abolished and she gained 6 lb. in weight. In the fasting condition and four to five hours after meals the blood sugar was normal—occasionally hypoglycaemic—but she had hyperglycaemia and glycosuria for one to three hours after carbohydrate meals, a condition comparable to that of a mild diabetic patient treated without insulin. P.I. (120 units) was given one morning, with 250 grams of carbohydrate during that day, and then no more insulin and only protein and fat food until the experiment ended—fifty-five hours after the dose of P.I.—by giving carbohydrate food. Details from the twenty-four to the fifty-eight hours (in Table II) show a complete control of glycaemia for fifty-five hours with slightly hypoglycaemic phases (no symptoms) and slight waves of higher blood sugar—which perhaps can be correlated with meals of protein and fat. Ketonuria recurred and persisted from the thirty-seventh hour; its significance will be discussed later.

It is thus clear that protamine insulinate can act not only twice as long as insulin, but even for more than two days.

Speed of Action of P.I.

If P.I. is absorbed more slowly and acts longer, the speed with which it exerts its action might be supposed to be slower than that of ordinary insulin. This was tested by a comparison of the effect of I. and P.I. on a high fasting blood sugar in Case I, the usual carbohydrate breakfast being withheld. Table III shows the

TABLE III.—Effect of P.I. and I. on a High Fasting Blood Sugar

Hours After Injection	Blood Sugar in mg. per 100 c.cm.		
	340	385	438
0			
1	250 (−90)	354 (−31)	387 (−51)
2	135 (−115)	270 (−84)	303 (−84)
3	57 (−78)	183 (−87)	201 (−102)
4	44 (−13)*	161 (−22)	163 (−38)
5	103 (+59)	150 (−11)*	126 (−37)*
6	138 (+35)	384 (+234)	346 (+220)
7	154 (+16)	350 (−34)	
8	180 (+26)	270 (−80)	
Amount Injected:	36 units of I.	36 units of P.I.	80 units of P.I.

* 50 grams of carbohydrate given.

fall of blood sugar and also the effect of giving ingested carbohydrate (50 grams of sugar) when insulin action was well established. In each case a similar breakfast of protein and fat (bacon and egg) was taken at hour 1 for the patient's comfort. The figures in brackets show the absolute fall and rise from hour to hour.

It is clear that I. is quicker in its action, although the difference is not as great as we had expected, since the action of P.I. on endogenous sugar is effective and not much behind that of I. But even at the height of its action P.I. is incomparably the poorer in dealing with ingested sugar. The effect of doubling the dose of P.I. produced little if any more rapid fall of blood sugar. At the beginning of each experiment the patient was excreting large amounts of ketones. These were rapidly and completely removed in three hours by I., and more slowly and only partially by P.I.

Control of Ingested Carbohydrate by P.I.

The above experiments show that when P.I. is controlling or even reducing endogenous sugar it deals remarkably poorly with ingested carbohydrate and fails to prevent a relatively enormous increase in blood sugar. The Danish workers^{1, 2} have recognized this, and in cases in which they give P.I. at night have been forced to give I. before a carbohydrate breakfast to obtain that full control of hyperglycaemia and glycosuria which is their ideal. We have tried, without observing much difference, in several other experiments to see whether P.I. at different stages of its absorption—that is, half an hour or four hours after injection—can obtain better control over the uniform 50 grams of carbohydrate used. It is very poor in this respect compared with I. An experiment in Table IV shows this clearly with large doses of prota-

TABLE IV

Time	—	Blood Sugar in mg. per 100 c.cm.	
		Arterial	Venous
8.30 a.m.	32 P.I.	132	—
10.0 a.m.	50 grams carbohydrate	102	101
11.0 a.m.		296	264
noon		278	275
1.0 p.m.		183	178

mine in full action. It also shows a comparison of simultaneous arterial and venous blood-sugar values, which are agreed to be an index of sugar utilization by the tissues.

After carbohydrate food in a normal person, or after injection of a large dose of insulin in a diabetic, the venous blood contains far less sugar than the arterial, and the arterio-venous difference should be as much as 20 to 80 mg. per 100 c.cm. We consider that the arterio-venous difference in this experiment is very slight, and reveals little evidence of peripheral activity of P.I. However, when large enough doses of protamine insulinate are given to maintain a very low blood sugar (40 to 60 mg. per 100 c.cm.) fairly liberal amounts of carbohydrate (50 grams a meal or 170 grams a day) have been well tolerated without a rise in blood sugar above 150 mg. and without production of glycosuria. We have been able to control Case I on a liberal amount of carbohydrate (170 grams) with two doses of P.I.: this patient had frequent bouts of glycosuria and persistent ketonuria with oscillating blood sugars on three doses a day of ordinary insulin.

The Occurrence of Hypoglycaemia

We have found, like other observers, that P.I. is much less likely than I. to cause hypoglycaemic symptoms at the same or even lower concentration of blood sugar, doubtless because the rapidity of the fall of blood sugar, as well as its absolute value, is an important factor. We have observed, however, one severe hypoglycaemic attack after P.I., while mild symptoms, at least, are apt to appear if big enough doses are given to attempt to control the hyperglycaemia and glycosuria which result from any reasonable carbohydrate meal. Such attacks occur many hours after carbohydrate meals, and are mostly to be expected between 3 and 6 a.m.

Clinical Conclusions

These clinical experiments amplify and confirm previous published experiences, and permit of the following conclusions.

1. Protamine insulinate acts more slowly and for a much longer period than ordinary insulin, and a large dose can act for more than two days.

2. It is much weaker in dealing with carbohydrate food and usually cannot prevent hyperglycaemia after meals. In contrast, its action on endogenous sugar is nearly as rapid and good as ordinary insulin.

3. It causes less symptoms of hypoglycaemia than ordinary insulin, even at the same blood-sugar concentration. Protamine insulinate is certainly absorbed more slowly from the subcutaneous tissues. It therefore has qualities which promise great use in severe diabetics with oscillating blood sugars and rapidly recurrent ketosis. On the other hand, it is not strong enough in its action to control ingested carbohydrate, so that the use of ordinary insulin before carbohydrate meals and protamine insulinate in the evening has been advocated and is necessary to obtain full control of the diabetic condition.

Another insulin preparation combining the qualities of ordinary and protamine insulinate would obviously be more useful than either, and it is not too much to hope that the Danish or other experimenters may soon obtain this. In the meantime, it must be stated that protamine insulinate, great though its advantage may be in some cases, is not generally obtainable for clinical use in this country, and is indeed still in the experimental stage.

A DISCUSSION OF INSULIN ACTION

BY

R. D. LAWRENCE

Deductions from Experiments with P.I.

From the above experiments with protamine insulinate we can distinguish two different phases of insulin action in the severe diabetic. A low concentration of insulin in the tissues, such as P.I. gives, is able to control hyperglycaemia from endogenous sources for long periods, but is quite incapable of coping with ingested carbohydrate as the higher concentrations of ordinary insulin do. I conclude that in normal carbohydrate metabolism a continuous small secretion from the pancreas (or perhaps merely a small residue from the last active secretion) controls the blood sugar continuously, but that a large extra secretion must be produced by the pancreas by the stimulus of ingested carbohydrate to deal with this successfully. Such an increased secretion of insulin in response to a rising blood sugar is a point of view which has been suggested before, but it has never been satisfactorily proved. Indeed, several experiments, especially recent ones by Soskin on depancreatized dogs, cast serious doubt on it.

Another most interesting observation, shown in detail in Table II, is the return of a heavy ketonuria from the thirty-seventh hour after P.I., at a time when the glycaemia was still completely controlled. During this time the patient was not in a typical diabetic condition, and the ketosis is comparable to that which ensues from carbohydrate starvation in normal individuals and is supposed to coincide with the exhaustion of liver glycogen. Whether her ketosis (occurring twenty-six hours after the last carbohydrate meal) coincided with glycogen depletion or was partly due to the protein and fat meals we cannot know for certain, but it did not develop until ten hours after the first protein and fat meal. This type of starvation ketosis is generally agreed to result from the accumulating by-products of protein and probably fat katabolism during the process of neoglucogenesis in the liver.