ANALYSIS OF THE ACCESSORY FACTORS IN THE CAUSATION OF DERMAL REACTIONS TO INSULIN

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Local reactions in the skin were observed soon after the introduction of insulin for the treatment of diabetes mellitus, and one of the first papers to record this phenomenon was by Joslin[1] and his co-workers in 1922. They stated that induration at the site of injections of insulin was frequent and described four cases of urticarial wheals with pruritus. One patient developed a small crusted ulcer without evidence of infection, and it was suggested that this might possibly be due to a burn from tricresol, the preservative used.

Subsequently many authors[2,3,4,5] have described the local sensitisation phenomenon to insulin therapy. In a comprehensive article published in 1932, Allan and Scherer[6] considered that the local reactions at the site of injection might be due to chemical irritation such as:—1. High concentrations of salts used in early preparations of insulin. 2. Excessive amounts of tricresol used as a preservative. 3. Acidity of early preparations. 4. Injection of denatured alcohol used for cleaning skin and storing syringe. On the other hand, these local reactions might be due to hypersensitivity to insulin and could be ascribed to:—5. Pancreatic protein of the animal from which the insulin was obtained. 6. Insulin protein itself.

When the first diabetic patients were treated with insulin and complained of stinging and local reactions at the site of injections, Banting *et al.*[3] thought that the high salt content of the pancreatic extracts was the cause. The salt content was reduced in subsequent preparations, but certain samples of this insulin continued to give rise to local skin sensitivity. In 1923, Banting, Campbell and Fletcher[7] used a "practically protein free" insulin preparation which gave rise to urticarial eruption in only one or two sensitive patients under their care. By modern standards the early preparations were extremely crude, and in 1925 Campbell and Mcleod[8] considered that local reactions were steadily diminishing in number with the increasing purity of the insulin.

Wilder *et al.*[2] also described the local effects of insulin therapy and recorded a few cases where necrosis and sloughing of the skin had occurred. This reaction was attributed to the preservative—tricresol—contained in the preparation. It is rare to observe necrosis of the skin following injections at the present time, and the local reactions described by Joslin *et al.*[1] and Wilder *et al.*[2] may have differed fundamentally from those encountered to-day. More recently Leavitt and Gastineau[9] stated that preservative in the insulin solution may cause local reactions, but they cited no patient with necrosis of the skin. Although many authors have stated that preservative in the insulin

solution appears to be the irritating factor, no carefully controlled experiment has been published demonstrating this effect.

The distinctly acid reaction of insulin solution has been implicated as the cause of local erythema. In 1924, Stillwell[10] described a method of neutralising the acidity with 6 per cent. solution of sodium bicarbonate and 3 per cent. of tricresol. By injecting this neutralising solution with the insulin the author claimed that local reactions in a previously sensitive patient were abolished. More recently Page and Bauman[11] in a detailed investigation used an acid control solution prepared from potassium acid phosphate which had *p*H 3·5, similar to that of globin insulin. They tested a series of diabetic patients with this solution and only obtained 1·2 to 3·0 per cent. of positive reactions.

It has been suggested that injection of denatured alcohol used in cleaning the skin or storing the syringe may cause erythema. Allan and Scherer[6] reported that skin irritation in one of their patients was due to hypersensitiveness towards the formalin contained in the alcohol. Storage and sterilisation of the syringe and needles as well as injection technique were carefully checked in all patients of the investigation. In none could the injection of denatured alcohol be implicated.

Many workers have investigated the protein impurities in insulin preparations. Campbell *et al.*[12] used crystalline insulin, which had a reduced animal pancreatic protein content, and stated that the induration of the subcutaneous tissues and area of reddening of the skin surrounding the site of injection was less than with commercial insulin. This work has been confirmed by many workers[13,14,15,16]. During 1949, Paley[17] investigated the effect of insulin recrystallised six times. A group of diabetic patients reacting locally to various brands of insulin were tested intracutaneously with insulin from their current vial and also with the recrystallised insulin. A striking reduction in the mean area of reaction was seen with the purified insulin.

Whilst the investigation left no doubt that the main factor causing local sensitisation was some substance closely associated with insulin itself, it was suggested that there may be accessory factors such as the *p*H of the solution and also the retarding substance, salmine sulphate. The present investigations were devised to ascertain the effect of preservative, cresol B.P., *o*-cresol and other constituents of commercial insulins on the local sensitisation phenomenon.

Experimental Method and Results

Twelve diabetic patients were selected because they were showing erythematous reactions to injections of all brands of insulin.

Each patient received intra-cutaneous injections of various test solutions on the flexor surface of the forearm. In the first experiment three solutions were used:—

A. Sterile water adjusted to *p*H 3·0 to 3·2.

B. Sterile water adjusted to *p*H 3·0 to 3·2 with 0·3 per cent. of cresol B.P.

C. Injection of commercial soluble insulin (made from crystalline material) *p*H of the solution was 3·0 to 3·2 and it contained 0·3 per cent. of cresol B.P.

The test dose consisted of 0·02 ml. of each solution, and the reactions were read after 15 minutes interval and graphed.

Using the technique of intracutaneous testing it was found that these sensitive diabetic patients reacted to buffer solution alone. Table I shows the mean area of reactions for these patients to this solution to be 317·5 sq.mm. This effect, however, is reduced by the addition of 0·3 per cent. of cresol B.P. The difference between the mean areas is markedly significant statistically when " Student's t " test is applied to the data.

TABLE I

INTRACUTANEOUS REACTIONS TO TEST SOLUTIONS

SOLUTION	REACTION MEAN AREA sq. mm.	DIFFERENCE	" t "
A. Sterile Water :— *p*H. 3·0 to 3·2	317·5	245·4	6·5 t=2·797 P=0·01
B. Sterile Water :— *p*H. 3·0 to 3·2 ... ... +0·3 per cent. of cresol	72·1		
C. INSULIN :— *p*H. 3·0 to 3·2 ... ... +0·3 per cent. of cresol	456·9	384·8	3·368 t=2·797 P=0·01

A significant increase in mean area of reaction is observed when solution " C " (commercial insulin) is compared with solution " B " (sterile water, *p*H 3·0 to 3·2 + 0·3 per cent. of cresol B.P.). When insulin is compared with solution " A " (sterile water *p*H 3·0 to 3·2) an increase in mean area is observed but this is not significant.

From the data presented in Table I, it would appear that the reduction in area of reaction caused by the addition of cresol B.P. to sterile water, *p*H 3·0 to 3·2 is nullified when insulin is added. Comparison of solutions " B " and " C " suggest that the increase in area of reaction is due to some substance introduced by the addition of insulin. The minimal response to solution " B " may be explained by the local anæsthetic action of cresol. Burfoot[15] in a personal communication stated that:—" Injections of insulin in America usually contain phenol as a preservative, although *o*-cresol is used by at least one firm. In Great Britain cresol B.P. or *o*-cresol is used. The reason for the use of *o*-cresol is probably due to it being a pure chemical substance whereas cresol B.P. is a variable mixture of the ortho, para and meta isomers." Although cresol B.P. does not appear to exert any action in the genesis of local insulin sensitisation, the following experiment was devised in order to determine any possible difference between the action of these two preservatives.

Two test solutions were prepared containing the same batch of insulin and identical with respect to *p*H, differing only in so far as one contained 0·3 per cent. of cresol B.P. and the other 0·3 per cent. of pure *o*-cresol as preservative.

A group of 15 diabetic patients exhibiting dermal reactions to various

brands of insulin were then tested intracutaneously using 0·02 ml. of each solution. Table II shows that insulin containing *o*-cresol gave a larger mean area of reaction than the same insulin containing cresol B.P. The difference is not statistically significant.

TABLE II

COMPARISON OF THE EFFECT OF CRESOL B.P. AND *o*-CRESOL

SOLUTION	MEAN AREA OF REACTION sq. mm.	DIFFERENCE	"t"
INSULIN + CRESOL (Solution C)	574	186	1·249
INSULIN + *o*-CRESOL	750		t = 1·055 when P = 0·3

In 1936, Hagedorn *et al.*[19] combined insulin with a basic protein substance (protamine-salmine) obtained from the sperm of rainbow trout. By adjusting the hydrogen ion concentration of this solution to that of tissue fluids they precipitated the protamine insulin. The preparation was used successfully in the treatment of a number of diabetic patients. Subsequently Root[20] and his co-workers confirmed that protamine zinc insulin exerted a prolonged hypoglycæmic effect. During the early days of treatment with the protamine insulins cutaneous reactions were said to be absent. Typical local sensitivity to injections of protamine insulin, however, was reported by Kern and Langer[21]. Fowler *et al.*[22] had presented a single report of a patient showing sensitivity to this type of insulin as early as 1937. The former investigators reported no positive reaction in any subject, diabetic or control when tested intracutaneously with a simple solution of protamine containing 0·1 mg. of nitrogen per ml. Their animal investigations also revealed an inability to sensitise guinea-pigs to protamine. Yet, Joslin[23] considers that local responses are more common since the introduction of protamine zinc insulin. In view of conflicting evidence implicating protamine as a cause of local skin reactions, further investigation was undertaken. Three test solutions were used:—1. A phosphate buffer solution at *p*H 7·0 to 7·2. 2. A 0·052 per cent. sterile solution of salmine sulphate adjusted to *p*H 3·0 to 3·2 which prevents precipitation. 3. Ordinary commercial zinc protamine insulin containing insulin from the same batch as solution C, the *p*H was 7·0 to 7·2 and salmine was identical with test solution "2."

Intracutaneous tests of these solutions were performed on the 12 sensitive patients at the same time as the injections of solutions "A," "B" and "C." The mean area of reaction to phosphate buffer solution at *p*H 7·0 to 7·2 was small and measured 122·6 sq. mm. This result was not unexpected since the *p*H of body tissues is of the same order. The 0·052 per cent. sterile solution of salmine sulphate was prepared at *p*H 3·0 to 3·2 in order to keep it in solution and thus prevent inconsistent injections of a suspension. If the mean area of reaction to this solution is compared with that obtained from solution "A" (sterile water adjusted to *p*H 3·0 to 3·2) they are found to be almost identical (Table III).

TABLE III

EFFECT OF SALMINE SULPHATE

SOLUTION	MEAN AREA OF REACTION sq. mm.	DIFFERENCE
SOLUTION "A" (Water *p*H 3·0 to 3·2)	317·5	5·7
SOLUTION "2":— (Water *p*H 3·0 to 3·2 +salmine sulphate)	323·2	

The findings presented here confirm Kern and Langer's[21] results that salmine does not cause skin reactions. Further indirect evidence is supplied by the mean area of reaction to intracutaneous test injections of commercial zinc protamine insulin (solution "3"). This was 430 sq. mm. and corresponds closely to the mean area of reaction obtained from test injections of commercial soluble insulin on the same series of patients, *viz.*, 456·9 sq. mm. (Table I).

In a previous communication Paley[17] demonstrated that the mean area of reaction to an intracutaneous injection of insulin recrystallised 6 times was 150·6 sq. mm. When this was compared with the mean reaction area obtained with test injections of different brands of insulin a significant reduction in area was observed.

The insulin recrystallised 6 times came from the same parent batch of insulin as solution "C" used in the present investigation. Concurrent tests were performed with solution "C" in the former experiment and a comparison, therefore, can be made with the highly purified insulin (Table IV).

TABLE IV

RESULT OF PURIFYING INSULIN

SOLUTION	MEAN AREA OF REACTION sq. mm.	DIFFERENCE
INSULIN:— (Solution C) 34 cases	388·1	237·5±52·9
INSULIN:— Recrystallised 6 times 34 cases	150·6	

It will be seen that there is a strikingly significant reduction in area of reaction to commercial soluble insulin when its insulin is recrystallised 6 times. This finding indicates that some "reacting factor" is removed during purification.

CONCLUSIONS

Intracutaneous testing in a large group of sensitive diabetic patients revealed some who gave reactions to substances other than insulin. Occasionally patients who did not react intracutaneously to the test insulin reacted to other constituents of their routine therapeutic insulin

solutions. Substances which gave rise most frequently to these reactions were sterile water, *p*H 3·0 to 3·2 and 0·052 per cent. salmine sulphate solution at same *p*H.

While aforementioned factors may operate in isolated cases, they did not appear to exert a significant effect on a series of sensitive patients. Cresol B.P. did not produce a reaction when injected intracutaneously in the strength supplied in commercial insulin, and no obvious advantage would appear to be gained by the use of *o*-cresol. Salmine sulphate when injected intracutaneously appears to be inert and cause no reaction. Comparison between commercial zinc protamine insulin and soluble insulin is important. No significant difference was shown between the mean areas for these two solutions when injected intracutaneously. Because of the widely differing composition of soluble insulin and zinc protamine insulin solutions, a direct comparison is not valid when investigating the mechanism of dermal reactions to insulin. Since preservative, salmine sulphate and *p*H do not appear to exert a dramatic influence on the skin, a similar mean area of reactions to both these "insulins" strongly supports the hypothesis that their common factor (insulin or closely associated substances) is the causal agent in the production of dermal reactions during insulin therapy. Stronger support for this hypothesis is provided by the marked reduction in reaction observed with highly purified insulin.

SUMMARY

1. A brief historical review is given of possible factors involved in the production of local cutaneous reactions to insulin.

2. Neither the *p*H of the solution when given with cresol B.P. nor salmine sulphate appears to exert any irritant effect on the skin.

3. No statistically significant difference is observed in the area of reaction when pure *o*-cresol is substituted for cresol B.P.

4. It has been shown that these accessory factors cause little or no irritation of the skin, and the main factor in local sensitisation is some substance closely associated with insulin.

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REFERENCES

1. Joslin, Gray and Root, *J. Metab. Res.*, 1922, **2**, 651.
2. Wilder, Boothby, Barborka, Kitchen and Adams, *J. Metab. Res.*, 1922, **2**, 701.
3. Banting, Campbell and Fletcher, *J. Metab. Res.*, 1922, **2**, 547.
4. Lawrence, *Lancet*, 1925, **1**, 1125.
5. Strauss, *Klin. Wchsch.*, 1925, **1**, 491.
6. Allan and Scherer, *Endocrinology*, 1932, **16**, 417.
7. Banting, Campbell and Fletcher, *Brit. med. J.*, 1923, **1** 8.

8. Campbell and Macleod, *Insulin*. The Williams and Wilkins Co., Baltimore, 1925, 77.
9. Leavitt and Gastineau, *Arch. intern. Med.*, 1947, **80**, 271.
10. Stillwell, *Brit. med. J.*, 1924, **2**, 465.
11. Page and Bauman, *J. Amer. med. Ass.*, 1944, **124**, 704.
12. Campbell, Gardiner and Scott, *J. clin. Invest.*, 1930, **9**, 28.
13. Baker, *Arch. intern. Med.*, 1936, **58**, 373.
14. Altshuler, *Ann. intern. Med.*, 1937, **11**, 901.
15. Ulrich, Hooker and Smith, *New Engl. J. Med.*, 1939, **221**, 522.
16. Jorpes, *Arch. intern. Med.*, 1949, **83**, 363.
17. Paley, *Lancet*, 1949, **256**, 1216.
18. Burfoot, *Personal communication*, 1949.
19. Hagedorn, Jensen, Krarup and Woodstrup, *J. Amer. med. Ass.*, 1936, **106**, 177.
20. Root, White, Marble and Stotz, *ibid.*, 1936, **106**, 180.
21. Kern and Langer, *ibid.*, 1939, **113**, 198.
22. Fowler, Bensley and Rabinowitch, *Canad. med. Ass. J.*, 1937, **36**, 561.
23. Joslin, *Treatment of Diabetes Mellitus*, Henry Kimpton, London, 1946, 465.