a certain length of time. I think, however, that these cases represent failures rather than proof of a diabetogenic action, as was the opinion of the reporting physician. We have no other reports of the diabetogenic action and I know of no animal experiments in which diabetes was caused by the compound.

Experimental findings are about the same as reported at the conference in March. More observations are published that BZ-55 acts even when β-cells are no longer present, as in complete alloxan-diabetes and in the depancreatized dog. At least, there is an action of BZ-55 if it is combined with insulin. It is also significant that the partially depancreatized dog easily goes into hypoglycemic shock when treated with BZ-55. It is difficult to reconcile this finding with the exclusive theory of the β-cell stimulation. There must be a peripheral mechanism and we find in the diaphragm of the rat in vitro an increased uptake of glucose but not an increased storage of glycogen by BZ-55 in therapeutic concentrations. On the other hand, Dr. v. Holt at the Institute of Prof. Kühnau has demonstrated hyperplasia of the islets in animals after long treatment with large doses of BZ-55. This is in agreement with the findings of the investigators in the United States and Canada. We have repeated the studies on the α-cells, especially when using chemically related substances. In a general concept of antidiabetic action the α-cells still cannot be neglected.

Finally we agree with Dr. Best that there is more than one point of attack in the body. We have to consider a peripheral, a β-cell and perhaps an α-cell action of BZ-55.

Metabolic Effects of Arylsulfonylurea Compounds in Normal Subjects and in Diabetic Patients

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In an effort to define the mode of action of sulfonylurea compounds our initial interest was directed at studies which sought answer to the following questions: (1) Do the sulfonylurea compounds suppress the pituitary-adrenal system? (2) Do the sulfonylurea compounds antagonize the peripheral effects of adrenal steroids? (3) Do the sulfonylurea compounds block the hyperglycemic effect of adrenalin and glucagon? (4) Do the sulfonylurea compounds increase sensitivity to exogenous insulin?

With these questions in mind extensive metabolic balance studies and numerous individual testing procedures have been performed before, during and following administration of BZ-55 and Orinase in normal and diabetic subjects.

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At the time of the Second Conference on Substance BZ-55 in March 1956, we presented data[1] which indicated that the sulfonylurea compounds: (1) do not suppress the pituitary-adrenal system, (2) do not antagonize the peripheral effects of adrenal corticoids, and (3) do not block the hyperglycemic effects of glucagon and adrenalin. No evidence of potentiation of insulin activity could be demonstrated. Thus the blood-sugar-lowering property of these compounds was thought to be via another mechanism.

Since March of 1956 further extensive studies have been performed before, during, and following administration of carbutamide and Orinase in normal men and in various types of diabetic subjects.[2]

In general, the results of these studies have been consistent with the earlier interpretations and therefore the details of these studies will not be presented at this time.

In a further effort to elucidate the mode of action of sulfonylurea compounds a group of acute experiments have been performed on *normal subjects* to determine the effects of administration of insulin, on the one hand, and of Orinase, on the other, upon blood levels of glucose, pyruvate and alpha-ketoglutarate. The results indicate that when hypoglycemia is produced by admin-

istration of insulin, the early changes in levels of blood pyruvate are opposite in direction from those observed when similar degrees of hypoglycemia are produced by Orinase[3].

METHOD OF INVESTIGATION

Eight insulin and nine Orinase experiments were performed. All subjects were given intravenously .05 units of glucagon-free insulin per kg. of body weight. In an additional experiment one subject received 0.1 units per kg. intravenously. Six subjects were given 1 gm. of sodium Orinase intravenously and one subject received 1.5 gm. intravenously. In addition, two subjects received 6 gm. of Orinase orally as a single dose.

During the intravenous tests blood samples for determination of glucose, pyruvate, and alpha-ketoglutarate were obtained at ten to thirty minute intervals. Levels of pyruvate and alpha-ketoglutarate were determined on whole blood by the paper chromatographic method of Seligson and Shapiro by Drs. Hennes and Wajchenberg.

RESULTS

Intravenous administration of insulin caused a mean fall in blood sugar of 48 per cent.

Also shown was the effect of intravenous administration of insulin on blood levels of pyruvate. In seven of eight experiments an increase in the level of blood pyruvate was the earliest change associated with hypoglycemia. In no instance did the level of pyruvate decrease before or during the time that blood sugar was falling which occurred within the first thirty minutes.

The intravenous administration of sodium Orinase caused a mean fall in blood sugar of 40 per cent.

The same experiments showed the effect of intravenous administration of sodium Orinase on blood levels of pyruvate. In contrast to administration of insulin, following intravenous administration of sodium Orinase, a decrease in level of blood pyruvate was the earliest change in five of seven experiments. This fall in level of pyruvate preceded the fall in blood sugar in two subjects. In only one experiment was the earliest change in blood pyruvate a significant increase within the first thirty minutes. Rises in blood pyruvate occurred after the first thirty minutes in several cases while the blood sugar was rising again and after symptoms of hyperadrenalemia had occurred.

It was during the first thirty minutes following intravenous administration of insulin and sodium Orinase that the most significant differences in blood pyruvate occurred in response to the two compounds. To illustrate that the differences in response of blood pyruvate are not dependent on greater or more rapid decrease of blood sugar following administration of insulin, the following findings are presented.

In two experiments we observed practically identical decreases of blood sugar produced by insulin and sodium Orinase in subjects T.S. and D.M. Nevertheless, the changes in levels of blood pyruvate were entirely dissimilar during the first thirty minutes. There was a marked rise in level of pyruvate following injection of insulin and a marked fall following administration of Orinase.

In subject D.W. we obtained a somewhat greater decrease in blood sugar following administration of sodium Orinase than following administration of insulin. The response to insulin was associated with a definite increase in level of blood pyruvate, while the initial response to Orinase was associated with a definite decrease in level of blood pyruvate.

There was no definite pattern of change in blood levels of alpha-ketoglutarate following intravenous administration of either insulin or sodium Orinase.

Finally, we showed that following oral administration of 6 gm. of Orinase the blood levels of pyruvate and alpha-ketoglutarate decreased at one hour and increased at two hours in each of two subjects. In one subject J.L. the decreases in blood levels of both intermediary metabolites preceded the fall in level of blood sugar and appeared fifteen minutes before symptoms of hypoglycemia. In both experiments the later increases of blood levels of pyruvate and alpha-ketoglutarate followed prolonged symptoms of hyperadrenalinemia.

SUMMARY

1. The acute changes in blood pyruvate and alpha-ketoglutarate associated with administration of insulin and of Orinase to normal subjects have been determined and compared.

2. The earliest change consisted of an increase in level of blood pyruvate associated with insulin-induced hypoglycemia, in seven of eight experiments. In none was there a decrease. In contrast, in seven of nine experiments the earliest change was a decrease in level of blood pyruvate when hypoglycemia was induced by Orinase.

CONCLUSIONS

The acute hypoglycemia following administration of insulin is usually associated with production of pyruvate in excess of its removal. On the other hand, acute hypoglycemia following intravenous or oral administration of Orinase is usually associated with removal of pyruvate

in excess of its production. These differences suggest that the immediate hypoglycemia induced by administration of insulin, on the one hand, and of Orinase on the other, occurs via different mechanisms.

These results do not support the concept that the sulfonylurea compounds produce acute hypoglycemia by stimulating rapid release of endogenous insulin.

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Group Discussion

FRANCIS D. W. LUKENS, M.D., (*Philadelphia*): One question about Dr. Anderson's report. I just wonder whether one can compare the results obtained at your time interval of a few minutes with those obtained when the blood sugar is lowered maximally by this drug in two to four hours. I just wonder whether you should have done the same experiment two to four hours after giving the drug?

GEORGE E. ANDERSON, M.D., (*Brooklyn*): We have done that in animals. In other words we have carried out investigations not only hours but days later. In the latter instance there *is* a depression. The methodology of these acute experiments will be thoroughly expounded in a coming issue of the *Journal of Clinical Nutrition.*

THOMAS H. MCGAVACK, M.D., (*New York City*): Regarding Dr. Achelis' paper, on what dosage level or levels were his patients carried for two and a half years without signs of toxicity?

J. D. ACHELIS, PROF. DR. MED., (*Mannheim-Waldhof, Germany*): One to two tablets or 0.5 — 1 gm.

LAURANCE W. KINSELL, M.D., (*Oakland, California*): In terms of peripheral effects and mechanisms, as we have reported previously, studies have been carried out in which patients have been maintained on constant diet containing essentially only fat. The objective was to produce major hyperketonemia and then to determine the possibility of modifying the hyperketonemia with particular reference to its progression. In studies that we have previously done, such a diet in any patient, diabetic or nondiabetic, without other measures being used, will proceed to marked ketoacidosis, and has to be interrupted because of this. In two such patients the administration of therapeutic amounts of the sulfonamide, either BZ-55 or on short-term experiments Orinase, has resulted in apparent major modification of this progression in that patients could be maintained indefinitely although with significant hyperketonemia but without ketoacidosis. This raises the question as to mechanism since we could know the total carbohydrates being metabolized from the urinary nitrogen on the one hand, and on the basis of the known glycerol moiety of the fat administered on the other. The question arose whether we might be having an increased oxidation of acetate peripherally as a result of BZ-55 administration. Two studies have been done using tracer doses of C^{14} carboxyl labeled acetate and determining its rate of appearance in expired CO_2 under controlled conditions. Our expectation was that there might be an increased amount of the C^{14} appearing in a stated period of time. The reverse has been found to be true. That is as far as we have gone.

HOWARD F. ROOT, M.D., (*Boston*): I would also like to ask Prof. Achelis a question, having recently had experience with one or two patients seriously ill with jaundice and other evidences of liver damage, particularly the type described by Dr. Duff some years ago. Now that the series has become so large, there will of necessity be patients who will die of other causes who have been receiving sulfonamides. Do you know whether any such patients have been studied with particular reference to the well-known pathologic findings in sulfonamide intoxication; that is, will there be an attempt to analyze, or is there anywhere any chance of getting some results of that sort? Two or three hundred thousand cases ought to offer a good many opportunities.

DR. ACHELIS: We have never seen jaundice in connection with our use of BZ-55. There have been patients on the drug with obstruction of the bile ducts. But in our reports there has been no jaundice resulting from the treatment.

DR. ROOT: I misunderstood you, Dr. Achelis. I assume you are saying that there have been some cases who, while receiving sulfonamides, have had jaundice which have been dismissed as jaundice not due to the