Group Discussion

JOSEPH IZZO, M.D., (*Rochester, New York*): Do I understand, Dr. Mary Root, that you have found no effect of carbutamide on liver glycogen?

MARY ROOT, PH.D., (*Indianapolis*): Our experiments are long-term ones, and if there is any effect at all, it is a decrease in liver glycogen after drug administration for one day to eight weeks. The liver is taken eighteen hours after the last dose. This is a different type of experiment from those previously reported to show increases in liver glycogen.

STEFAN S. FAJANS, M.D., (*Ann Arbor*): In relation to Dr. Lukens' paper, at the March conference we reported on a patient with diabetes mellitus and concomitant partial panhypopituitarism. A thirty-year-old male with craniopharyngioma failed to grow and also showed evidence of complete hypothyroidism and partial adrenal insufficiency. This individual is extremely sensitive to insulin. In this case BZ-55 had no consistent effect on the blood sugar, an observation in agreement with the experiment on the Houssay animal.

Again in correlation with Lukens' failure to find increased sensitivity to exogenous insulin, we could demonstrate none in our case following large amounts of BZ-55, although blood levels were 20 mg. per 100 ml. or more.

In relation to Dr. Vaughan's studies, both in normal and diabetic patients who respond to the sulfonylureas, we found no differences in the hyperglycemic effect to adrenalin and glucagon whether these substances were given by themselves or with BZ-55 and Orinase.

ROBERT H. WILLIAMS, M.D., (*Seattle*): With reference to Dr. Lukens' discussion, we have carried out studies with hypophysectomized and adrenalectomized rats. As in his studies with cats, we found that there was a normal hypoglycemic response to carbutamide in the hypophysectomized animal whereas there was a markedly accelerated response in the adrenalectomized rats.

M. E. KRAHL, PH.D., (*Chicago*): I would like to comment briefly on Dr. Lukens' and Dr. Vaughan's papers. These seem to me to emphasize the problem of the mechanism of action of these drugs.

If these observations of Lukens on the hypophysectomized cat are substantiated as they seem to be from Dr. Williams' comments, an explanation of a carbutamide effect based on either inhibition of insulinase or discharge of insulin from the pancreas would be ruled out.

In commenting on Dr. Vaughan's paper, and in general on effects on liver output of glucose produced by carbutamide, I ask the question: If the effect in the normal animal is due to an inhibition of glucose output, why is this not seen in a diabetic animal as well?

LAURANCE W. KINSELL, M.D., (*Oakland*): In relation to Dr. Lukens' paper, we have until now refrained from using carbutamide or tolbutamide in our totally hypophysectomized diabetics because of the precarious state of their vasculature and the feeling that we were not justified until the toxicity picture became clearer. We have, however, used carbutamide in one patient who is less than totally hypophysectomized but who, nonetheless, has evidence of some pituitary insufficiency. This man had received carbutamide before and after hypophysectomy. In general, he had some modification of insulin requirement on both occasions, but there was no significant difference posthypophysectomy as compared to prehypophysectomy. This also would confirm Dr. Lukens' observations.

R. E. HAIST, M.D., (*Toronto*): I should like to ask Dr. Lukens and Dr. Krahl if they think the absence of an effect in the hypophysectomized animal would preclude an influence on the pancreas mediated through pituitary or some other gland.

DR. KRAHL: It may not be a direct effect.

DR. MARY ROOT: In relation to Dr. Lukens' and Dr. Williams' results, Dr. Houssay reported at the Physiological Congress this summer that he also had found that there was no increase in insulin sensitivity in the hypophysectomized animal whereas there was an increase in the adrenalectomized animal. Houssay stated that you can save the adrenalectomized animal by giving tremendous amounts of glucose. But he asked: "Where is this glucose going?"

FRANCIS D. W. LUKENS, M.D., (*Philadelphia*): Dr. Fajans and Dr. Kinsell point out that man appears to behave like our experimental animal; Dr. Williams has given confirmation of this. The story I presented was a preliminary one, but perhaps might stand up as a physiological finding.

Dr. Krahl asks: "Why, if these drugs inhibit the discharge of glucose from the liver in the normal, don't they do so in the diabetic?" Two reasons occur to me on purely theoretical grounds: These drugs will act on a liver only when it is conditioned to a certain rate of metabolism. One might use that same argument, in the hypophysectomized animal for example. The liver, op-

erating at a minus 30 BMR, is so depressed it cannot respond further.

GEORGE ANDERSON, M.D., (*Brooklyn, New York*): A great difference in the calculation of the liver glucose output would depend on where the blood sample is taken. If you take it from the periphery it would not reflect change in the output of glucose by the liver. If it is taken directly from the hepatic vein the estimation would be more valid.

We have found that if one studies the hepatic vein flow directly, one can determine a decreased output by comparing that with the vena caval blood. In other words, if there is the same output into the hepatic vein as occurred before sulfonylureas were given, that should be preliminarily higher because it represents the arterial side. We have been finding that there is a decreased output into the hepatic vein even in the diabetic animal, even though this cannot be detected by the ordinary blood sugar methodology.

DR. LUKENS: I agree with Dr. Anderson that it's very important to measure it that way.

DR. IZZO: With reference to Dr. Krahl's question on glucose output: Does anybody have any information on the glucose output and the effect of this on liver slices in diabetic animals?

UNIDENTIFIED: There is no difference in glucose production.

DR. WILLIAMS: I want to comment on the inhibition of the degradation of insulin by these drugs when used in the usual therapeutic dosage. There is no question, as Dr. Vaughan indicated, that if one uses large quantities of these sulfonylureas in vitro there is marked inhibition of the degradation of insulin. Furthermore, this fact can be demonstrated by incubating the liver enzyme preparation with insulin with these inhibitors, and then testing various mixtures in rats, noting the degree of lowering of the blood sugar. There is no doubt that these sulfonylureas, when incubated in vitro with insulinase, will spare the degradation of the insulin.

We carried out two further types of experiment. The sulfonylureas were first given to the animal by gavage, in doses of 300 or 500 mg. per kg. These doses were definitely hypoglycemic. After a period of two hours, the liver was removed; on incubation in vitro no inhibition of the degradation of insulin was demonstrated. If all of the compound that we gave were in the liver, it still would not inhibit the degradation of insulin if one judges from the concentration that was found necessary in our in vitro studies.

In another experiment, we gave labeled insulin intraperitoneally to baby rats, and simultaneously gave the sulfonylureas by gavage or intravenously. We could not demonstrate any inhibition of the degradation of I^{131}-insulin. These conclusions are not in accord with those of Dr. Mirsky. There were some differences in technic but I would be inclined to conclude that, in the doses that are usually used therapeutically in diabetics, inhibition of degradation of insulin does not seem to be significant.

EARL W. SUTHERLAND, JR., M.D., (*Cleveland*): I'd like to ask Dr. Mary Root about the glycogen values because this bears on the mechanism of action. This is one place where the action of these compounds differs from the action of insulin. I wonder if it isn't because of the length of time after drug administration. As I recall, you reported some time previously the effect of glucagon on prolonged administration and found some hours after giving glucagon actually an increase in glycogen. I wonder if that result, and this finding of decreased glycogen may have something to do with the time period of sampling. I understood that you sampled as long as eighteen hours after the last dose.

DR. MARY ROOT: Yes, I sampled approximately eighteen hours after the last dose. Certainly there was a difference in the experiment in which we gave the animals insulin. The liver glycogen values were about the same as those in the controls, whereas the ones that had had carbutamide alone or carbutamide plus insulin had low liver glycogen values. These findings probably fit in with the time factor. It's going to take an experimental animal such as a rat to do this because the actual variation from animal to animal in the liver glycogen concentrations is so great in dogs, that since we only run three or four we don't really get statistically significant results on account of the small changes that occur.

DR. SUTHERLAND: One other comment regarding the liver slice and glucose output perhaps should be saved till later, but we find different results depending on the dose level. We do need to go to fairly high dose levels before we see these effects in liver slices—much like Dr. Vaughan. When concentrations are at a level around 1×10^{-3}, close to the therapeutic range, we see very little effect on glucose output by the liver slice.

MARTIN G. GOLDNER, M.D., (*New York*): In connection with Dr. Williams' comments on insulin degradation, Dr. Sol Berson in our laboratory from his studies is convinced that in therapeutic doses of the sulfonylureas there is no evidence of inhibition of insulin degradation. Neither is there, as Dr. Berson assures me, any inhibition of glucagon inhibition.