Reports

Carbutamide and Plasma Insulin Activity

For about two years, arylsulfonylurea derivatives—for example N-*p*-aminobenzene-sulfonyl-N′-*n*-butyl-urea (Carbutamide, *1*), have been used in the therapy of human diabetics. It is supposed by different authors that an inhibition of glucagon production represents the mode of action of Carbutamide (*2*). However, the morphologic appearance of the islets of Langerhans, as well as the known metabolic effects produced by Carbutamide drug, is not in agreement with such a mechanism of action (*3–5*). Whereas α-cell destruction by Synthalin or *p*-aminobenzenesulfonamide-5-isopropyl-1,3,4-thiodiazole (IPTD) in alloxan-diabetic animals causes a normalization of the metabolic disturbance (*6*), Carbutamide does not influence the hyperglycemia and glycosuria of alloxan-diabetic rats. The absence of a curative effect of Carbutamide in alloxanized (*3, 5, 7*), pancreatectomized (*8*), metasteroidal (*4*), or metahypophyseal (*8*) animals and the effectiveness of Carbutamide in normal or idiosteroidal rats (*3, 5*) point to the probability that the endogenous production of insulin may be indispensable for the blood sugar-lowering effect of the substance.

Previous experiments have shown that, under the influence of Carbutamide, the β-cells of the islets of Langerhans exhibit morphological changes indicating an increased activity of this system (*3–5, 9*). In long-term experiments, this stimulation results in a relative β-cell insufficiency as manifested by an impaired sugar tolerance in rats and dogs. In these animals, an increased diameter of β-cell nuclei and an enlargement of the islets with degranulation and occasional hydropic degeneration of the cells may be observed (*5*). In addition, Carbutamide causes a stimulation of the adrenal cortical activity which is indicated by a moderate "progressive transformation" (Tonutti, *10*) of the cortical zones, as well as by depletion of ascorbic acid (*3, 5*).

A stimulation of the insulin production is supposed to be followed by an increased level of blood insulin. Therefore, insulin activity of plasma in animals that had been treated with Carbutamide was investigated. Insulin assays were performed by the method of Randle (*11*). Instead of determining the glucose uptake of the isolated diaphragms, we measured the increase of glycogen synthesis, using the anthrone method of Seifter *et al.* (*12*).

The results (Table 1) show that Carbutamide produces a highly significant increase of insulin activity in normal animals, corresponding to the decrease in blood sugar level after a single dose of Carbutamide. In rats treated daily with the drug over a period of several months, no increase in plasma insulin activity was observed, according to the lack of blood sugar-lowering effect of the substance after continuous administration. The increase in muscle glycogen synthesis produced by insulin is not seen when Carbutamide is added to diaphragm *in vitro* (*13*). Therefore, the insulinlike effect of plasma of Carbutamide-treated rats on diaphragm glycogen cannot be attributed to a direct action of Carbutamide on diaphragm.

Our results indicate a stimulating effect of Carbutamide on insulin activity in short-term experiments. In addition, it was observed that continued stimulation of the β-cell system by Carbutamide is followed by a relative insufficiency of these cells, expressed by a lack of additional insulin output and the previously described decrease in sugar tolerance (*5*).

Besides certain morphological signs of enhanced β-cell activity (*3–5, 9*) the islets of rats that are repeatedly treated with Carbutamide or Tolbutamide (N-toluene-sulfonyl-N′-*n*-butyl-urea, *14*) (500 mg/kg daily for 3 weeks orally) exhibit an increased mitotic rate of β-cells.

The increase of insulin secretion described in a previous paragraph cannot fully account for the metabolic effects produced by Carbutamide and Tolbutamide. The lack of difference in the response of normal and hypophysectomized rats to the blood sugar-lowering activity of sulfonylurea derivatives (*15, 16*), as well as their insulin-sparing effect in pancreatectomized dogs (*8*) or alloxanized rats (*5*), and the increase of

Table 1. Plasma insulin activity (milliunits in 0.5 ml) and blood-sugar decrease (milligram percent) in Carbutamide-treated rats. In every group, 2-ml portions of plasma of two rats (male albino Sprague-Dawley rats of weight 280 to 330 g) were pooled, and the insulin activity was tested with four or five hemidiaphragms. Blood sugar estimations in plasma donors were performed before the ingestion of the drug and at the time of insulin determination. The significance of differences in insulin content was as follows: groups I and II, $P = 0.001$; groups I and III, no difference.

Group	Plasma donors	Mean value of approximate insulin activity (milliunits in 0.5 ml)	Standard deviation of insulin activity	Mean value of blood-sugar decrease (mg %)
I	24 normal rats fasted 2 hours	14.6	19.3	5
II	14 rats, 2 hours after a single oral ingestion of Carbutamide (300 mg/kg)	83.4	58.2	36
III*	20 rats, treated daily with Carbutamide orally (10 rats: 200 mg/kg; 10 rats: 300 mg/kg) for 3 months, 2 hours after administration (300 mg/kg)	14.8	21.3	8

* Before this period, 10 of these 20 animals had been pretreated for another 6 months with 25 to 100 mg of Carbutamide per kilogram, per day.

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liver glycogen level in fasting animals after administration of Carbutamide and Tolbutamide (*3, 15*), point toward mechanisms that are independent of the stimulating effect of these oral antidiabetics on insulin output.

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References and Notes

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Transport of Glucose by the Renal Tubule Cells of Anesthetized Dogs

Under ordinary circumstances the removal of glucose from the urine in the proximal tubules of the kidney is sufficiently complete to prevent the escape into the bladder of any significant fraction of the glucose crossing the glomerular barrier. The glucose moiety absorbed by the proximal tubule is usually considered to combine reversibly with a postulated intracellular carrier and to be returned to the peritubular fluid and thence to the peritubular capillaries and the renal vein. It is generally tacitly assumed that the transport process does not involve breakdown and resynthesis of the six-carbon chain of glucose. It has been suggested as an alternate hypothesis that the transport might involve a three-carbon unit (*1*). Evidence is presented here to substantiate the assumption that the six-carbon chain remains intact during the transport process (*2*).

The procedure is similar to that described in an earlier paper (*3*). Injection of a solution containing the blue dye, T-1824, glucose labeled with carbon-14 in the 1 position, and creatinine is made nearly instantaneously into the renal artery of an anaesthetized dog. Thirty successive samples of renal venous blood are obtained by means of a polyethylene catheter introduced into the left renal vein by way of the inferior vena cava. The concentration of each injected substance, determined in each blood sample, is divided by its concentration in the mass injected. The resultant concentration ratios are plotted as ordinates against sample number or time after injection as abscissas. The results of a representative experiment are shown in Fig. 1 (top).

That the radioactivity in the venous blood samples was derived from glucose rather than from other nonvolatile carbon compounds was established as follows. Paper chromatograms were prepared of desalted perchloric acid filtrates of the blood samples. Glucose was identified by means of aniline phthalate. Radioautograms and strip scanning for radioactivity indicated that glucose was the only substance present that possessed significant radioactivity.

The difference between the glucose and creatinine curves is assumed to represent the glucose absorbed from tubular urine by the proximal tubule cells and delivered to the peritubular capillaries. A necessary corollary of this assumption is that phlorhizin should, by blocking tubular transport of glucose, obliterate the differences between the glucose and creatinine curves. That it does this is shown in Fig. 1 (bottom). The broken line in Fig. 1 (top), relating the differences between the glucose and creatinine ratios, may therefore be taken to represent glucose returned to the circulation by the tubule cell.

Degradation, by a microbiological procedure (*4*), of the glucose in the venous blood samples containing this returned glucose showed that essentially all the recovered radioactivity resided in the 1 position. This absence of randomization establishes that there is no breakdown and resynthesis of the six-carbon chain of glucose in its transport across the tubule cell.

The mean transit time (*5*) from the glomerulus to the peritubular fluid cell border of glucose transported by the tubule cells is calculated as the difference between the mean transit times, from renal artery to catheter tip, of the transported glucose and of the creatinine; the assumption is made that creatinine does not enter these cells from either cell border. The average of the values found in eight experiments is approximately 10 sec. If it is assumed that the mean transit time of glucose from the glomerular barrier to the lumen border of the proximal tubule cells is of the order of 5 sec, then the mean transit time of glucose across the tubular cells is approximately 5 sec. The assignment of the value of 15 μ to the thickness of the cells and the assumption that diffusion is the mechanism of the passage of the complex across the cell permit calculation (*6*) of a value for the diffusion coefficient in the cytoplasm of the postulated glucose-carrier complex. This value is 0.2×10^{-6} cm²/sec. If it is now assumed, as was previously done for *p*-aminohippurate transport (*6*), that

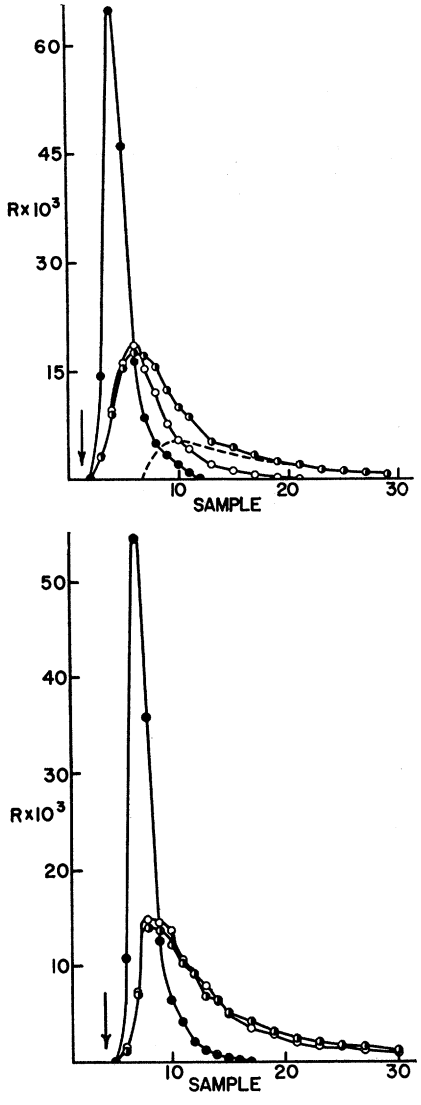

Fig. 1. Ordinates represent concentration of substance in sample divided by concentration in injection mass. Abscissas are the sample number. Filled circles indicate T-1824, open circles creatinine, half-filled circles glucose-1-C^{14}. Arrows denote the moments of injection. (Top) No phlorhizin; broken line indicates glucose returned from tubule urine; collection time, 1.32 sec per sample; (bottom), after phlorhizin; collection time, 1.40 sec per sample.