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ΑΝΑΚΟΙΝΩΣΙΣ ΜΗ ΜΕΛΟΥΣ

ΦΑΡΜΑΚΟΛΟΓΙΑ.— **Experiments on the action of Adipose Tissue Extracts (ATE) on Blood Glucose Concentration** *, *by Harry N. Antoniades* **. Ἀνεκοινώθη ὑπὸ τοῦ Ἀκαδημαϊκοῦ κ. Γ. Ἰωακείμογλου.

Earlier studies suggested that insulin in the blood of diabetic patients of the maturity-onset type circulates primarily as a high molecular weight inactive metabolite, which was called bound insulin (**1 - 3**). It was suggested that in thesse patients diabetes may result not only from a lack of endogenous insulin but also from a malfunction of the mechanisms regulating insulin activity (**2 - 3**). Such a malfunction may cause increased transformation of pancreatic active, free insulin into the inactive bound from by the liver and possibly other extrapancreatic tissues of these patients and a decrease in the rate of activation and utilization of their circulating bound insulin (**2 - 4**). The observation that adipose tissue extracts (ATE) can trigger *in vitro* the activation of bound insulin, in the presence of isolated muscle (**5 - 6**), suggested the possibility that ATE could also tripger *in vivo* the activation of circulating bound insulin. Such an *in vivo* activation of bound insulin by ATE could lead to an increased glucose utilization and therefore a decline in the blood glucose concentrations. Recent studies have shown

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* Χ. Ν. ΑΝΤΩΝΙΑΔΗ, **Πειραματικαὶ ἔρευναι περὶ τῆς ἐνεργείας ἐκχυλισμάτων λιπώδους ἱστοῦ (ATE) ἐπὶ τῆς πυκνότητος γλυκόζης εἰς τὸ αἷμα.**

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that ATE administration can indeed produce hypoglycemia *in vivo* in adrenalectomized rats (**7**).

The present studies demonstrate that ATE can produce a significant decline in the blood glucose concentrations of intact, spontaneously diabetic mice of the KK strain. The KK mice are considered to represent an inbred strain of diabetic animals with features characterizing human diabetes mellitus of the maturity-onset type (**8 - 10**). Both short-term and long-term studies with ATE in these animals are reported. Data are also presented on the hypoglycemic effects of homologous rat and heterologous bovine ATE in adrenalectomized rats following intraperitoneal or intravenous administration. Other studies describe the effect of ATE on circulating free and bound insulin in the blood of adrenalectomized rats and the distribution of glucose carbon into the glycogen and fat of various tissues of these animals following ATE administration.

MATERIAL AND METHODS

Preparation of partially purified adipose tissue extracts: rat ATE. Rat epididymal adipose tissue pads were homogenized in cold distilled water (about 2 ml of H_2O per g tissue) in a teflon grinder. The mixture was filtered through a gauze and a nylon cloth. Cold ethanol was added to the filtrate to a final concentration of 60 %. The mixture was placed at 2° C. The supernatant fluid was diluted with distilled water to an ethanol concentration below 8 % and was lyophilized. The dry powder was collected, dissolved in H_2O (about 20 mg dry powder per ml H_2O) and heated at 100° C for 5 minutes. The mixture was cooled and centrifuged (3,000 r.p.m.) at room temperature. The supernatant fluid was ultrafiltered through an Amicon UM-2 filter which is reported to allow the passage of substances with a molecular weight below 1,000, and the filtrate was lyophilized. One mg of the final preparation represented 30 - 40 g of epididymal adipose tissue.

Bovine ATE. Bovine mesenteric or perirenal adipose tissue was homogenized at room temperature with 60 % ethanol in a Waring blender. Two to three ml of ethanol solution were used per g wet tissue. The homogenate was filtered through a nylon cloth and the mixture was placed at 2°- 5° C overnight under continuous stirring. The mixture was then centrifuged at 2°- 5° C. Two volumes of acetone were added per

volume of supernatant fluid and the mixture was placed for 72 to 96 hours at —5° C. The clear supernatant fluid was siphoned off at —5° C with care not to disturb the fine precipitate collected in the bottom of the container. The final mixture was centrifuged at —5° C, and the precipitate was lyophilized. The dry powder was dissolved in a small volume of cold distilled water (about 20 mg dry powder per ml H_2O) and heated at 100° C for 5 minutes. The mixture was cooled and centrifuged at room temperature. The precipitate was discarded. Portions of the supernatant fluid were placed in individual vials and the content was lyophilized Each vial contained 10 to 15 mg of dry powder, representing about 300 to 450 g of wet tissue. Batches of 1 kg to 400 kg of bovine mesenteric fat were processed with the above technique.

The rat and bovine ATE preparations were devoid of insulin as measured by the double antibody radioimmunoassay technique of Morgan and Lazarow (**12**) as modified by Soeldner (**13**) (less than 5 microunits per mg ATE), and they did not exhibit biologic activity on isolated rat hemidiaphragm (less than 10 microunits per mg ATE).

Diabetic KK mice, fed ad libitum on Purina chow or fasted for 4 hours, were injected intraperitoneally with 1 ml of 0.15 M NaCl with or without ATE. Blood samples (0.05 ml in 0.95 ml 0.15 M NaCl) were collected at various intervals during a 4-hour period and their blood glucose concentrations were determined by a Technicon Autoanalyzer.

Short-term studies in KK mice were carried out both in our laboratories and at the Smith, Kline and French (SKF) Research Laboratories. The KK mice were supplied to us through the courtesy of Dr. James H. Birnie of SKF. The initial blood glucose levels of these animals were between 90 to 100 mg %. The study at the SKF Research Laboratories was conducted with an identical ATE preparation supplied by our laboratory. The initial blood glucose levels of the KK mice used at the SKF Laboratories were between 102 to 221 mg %.

Long-term studies in KK mice were carried out for a period of 15 days. Eight animals were treated with ATE and six animals with control saline. All animals were fed ad libitum on Purina chow during the experiment. Blood samples were collected daily in the morning before injection and at 4, 24 and in some cases 30 hours after injection. Blood glucose concentrations were determined by a Technicon Autoanalyzer.

Charles River Laboratory male adrenalectomized rats, weighing between 120 - 130 g, were injected a week after adrenalectomy. Each rat received intraperitoneally, or intravenously into the jugular vein 1 ml of 0.15 M NaCl with or without ATE. The food was removed from the cages of these animals before injection. During intravenous or intraperitoneal injection, the rats were narcotized lightly with 50% O_2 : 50% CO_2. Blood samples (0.1 ml in 0.9 ml physiological saline) were collected from the tail of the animals before the ATE administration and at various intervals after the administration. Blood glucose concentrations were determined by a Technicon Autoanalyzer. Special care was taken in each experiment to select animals with body weight within $\pm$ 2 g and with initial blood glucose levels varying no more than $\pm$ 5%.

In other studies, glucose - U - C^{14} (2 μC per rat) with and without bovine ATE (4 mg/rat) was injected intraperitoneally into adrenalectomized rats (6 animals in each group). Two hours after injection the rats were narcotized lightly with 50% O_2 : 50% CO_2, and the two hemidiaphragms, the two epididymal adipose tissue pads and liver pieces removed from the animals, weighed and processed for the extraction of glycogen or fat (**14 - 15**). The radioactivity in the glycogen precipitate of the muscle, liver and adipose tissue and the fat of adipose tissue was counted and expressed as CPM per g wet tissue.

Blood samples were collected from adrenalectomized rats three hours after intraperitoneal injection of bovine ATE (4 mg/rat) or control saline. The blood was allowed to clot and the serum separated by centrifugation. Duplicate serum samples (0.2 ml) were immunoassayed for free insulin. The remaining serum samples were pooled and their bound insulin was extracted by resin adsorption and elution as described elsewhere (**11**). The bound insulin preparations thus obtained were then lyophilized, dialyzed against Gey and Gey bicarbonate buffer and assayed for insulin activity *in vivo*, in intact fed rats by the intraperitoneal assay (**14 - 15**). Pooled sera obtained from KK mice four hours after the administration of bovine ATE (4 mg/mouse) or control saline were immunoassayed for free insulin. A portion of the whole sera of these animals was also assayed for insulin activity *in vivo* by the intraperitoneal assay.

RESULTS

Adrenalectomized rats. The effects of partially purified homologous rat epididymal ATE and heterologous bovine perirenal ATE in adrenalectomized rats are shown in Figure 1. Hypoglycemia in these animals

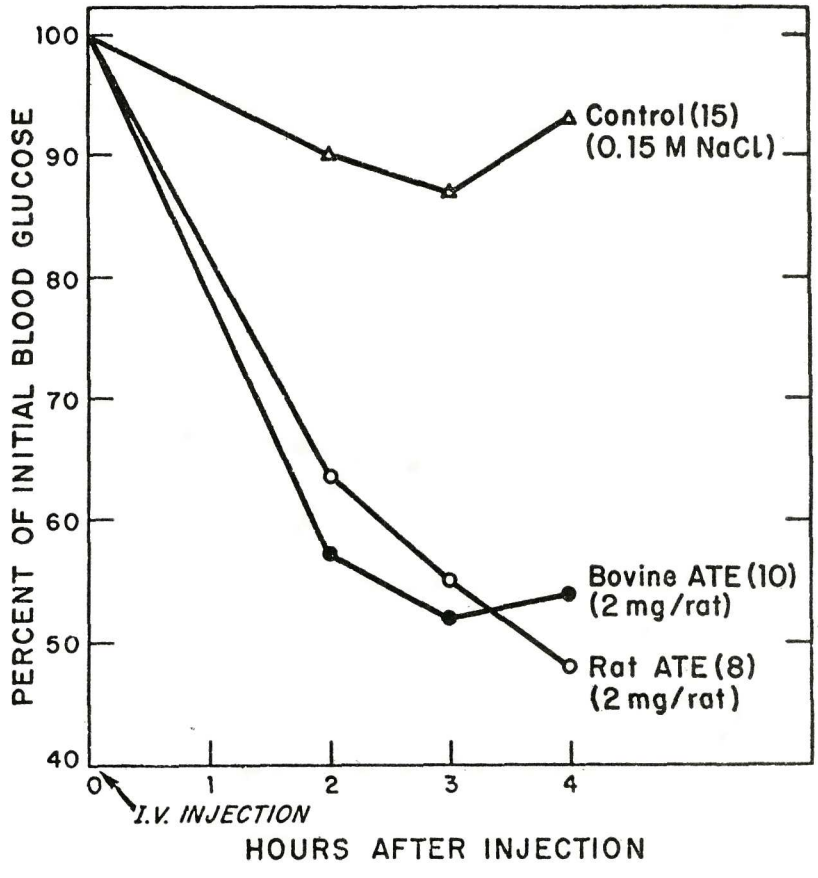

Fig. 1.— Effects of partially purified rat epididymal and bovine perirenal ATE on the blood glucose concentrations of adrenalectomized rats. Each animal was injected intravenously with 1 ml of 0.15 M NaCl with and without ATE. Values represent means ± SEM. (Initial blood glucose concentration : 83.6. ± 4.5 mg %).

was produced following intravenous or intraperitoneal administration (Figure 2).

Spontaneously diabetic KK mice. Short-term studies: The effect of a single administration of ATE in fasted (4-hour) or fed diabetic KK mice

is shown in Figures 3 and 4. Administration of partially purified bovine mesenteric ATE produced a significant decline in the blood glucose concentrations of these animals compared with mice injected with control

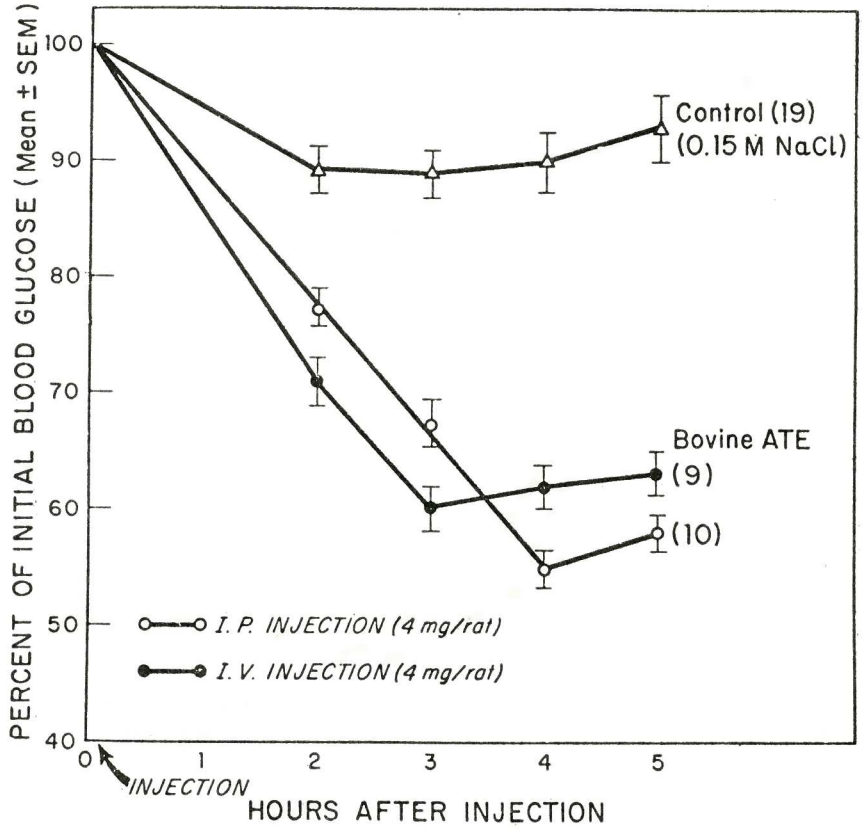

Fig. 2.— Effect of partially purified bovine mesenteric ATE on the blood glucose concentrations of adrenalectomized rats following intravenous or intraperitoneal administration. (Initial blood glucose concentration : 86.4 ± 3.7 mg %).

saline solution. The data with ATE in fasted KK mice were obtained independently in our laboratory (Fig. 1, Exp. I) and at the SKF Research Laboratories (Fig. 1, Exp. II). The results of these two sets of experiments are similar. In both fed and fasted animals the blood glucose concentrations dectined progressively with time following ATE administration. A mild decline was also observed in the blood glucose concen-

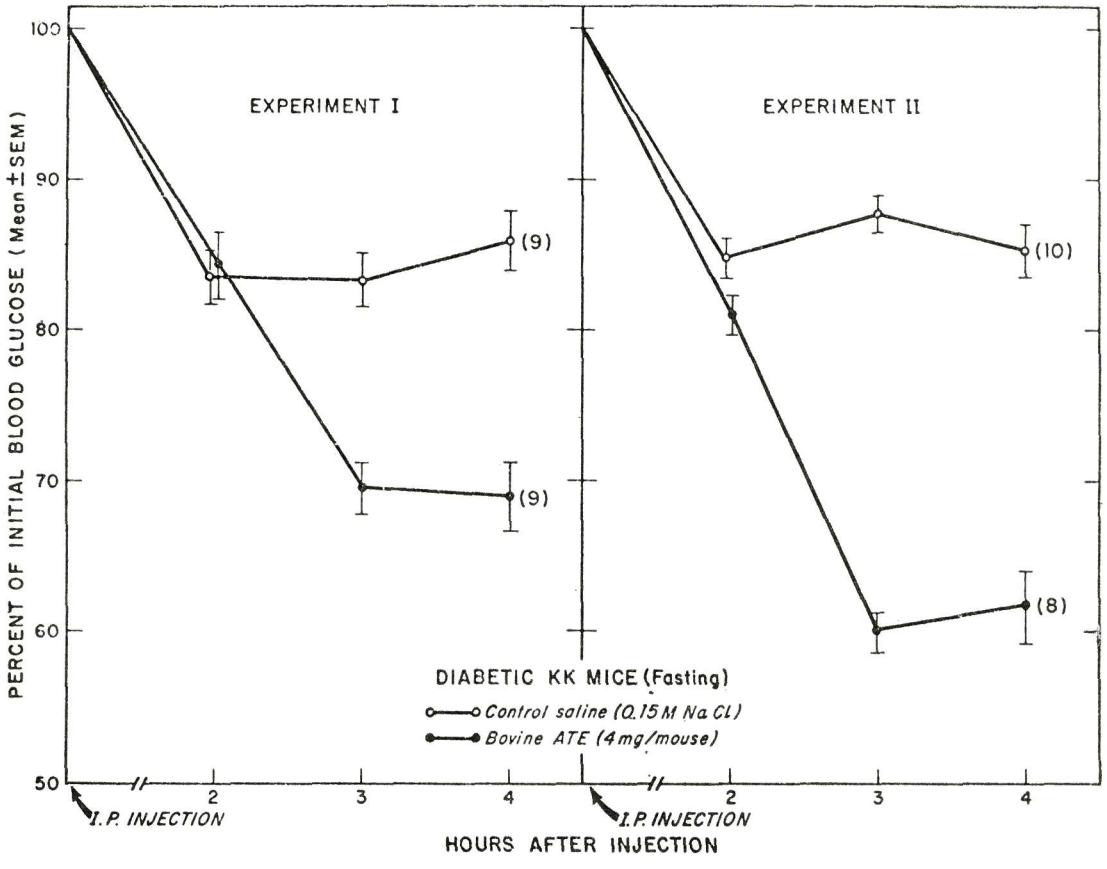

Fig. 3.—Effect of partially purified bovine mesenteric ATE on the blood glucose concentrations of fasted (4-hour), intact, spontaneously diabetic mice of the KK strain. Each animal was injected intraperitoneally with 1 ml of 0.15 M NaCl with and without ATE. Exp. I was carried out in our laboratories and Exp. II at the Smith, Kline and French Research Laboratories. (Initial blood glucose concentration: Exp. I, 98 ± 2.2 mg %; Exp. II, 163 ± 9.9 mg %).

trations of fasted animals injected with control saline. This may be due to the 4-hour fasting of the KK mice before injection. The blood glucose concentrations of fed animals injected with control saline remained unaffected (Figures 4 and 5).

Long-term studies in KK mice are presented in Fiqure 5. ATE administration produced a prolonged effect in the blood glucose concen-

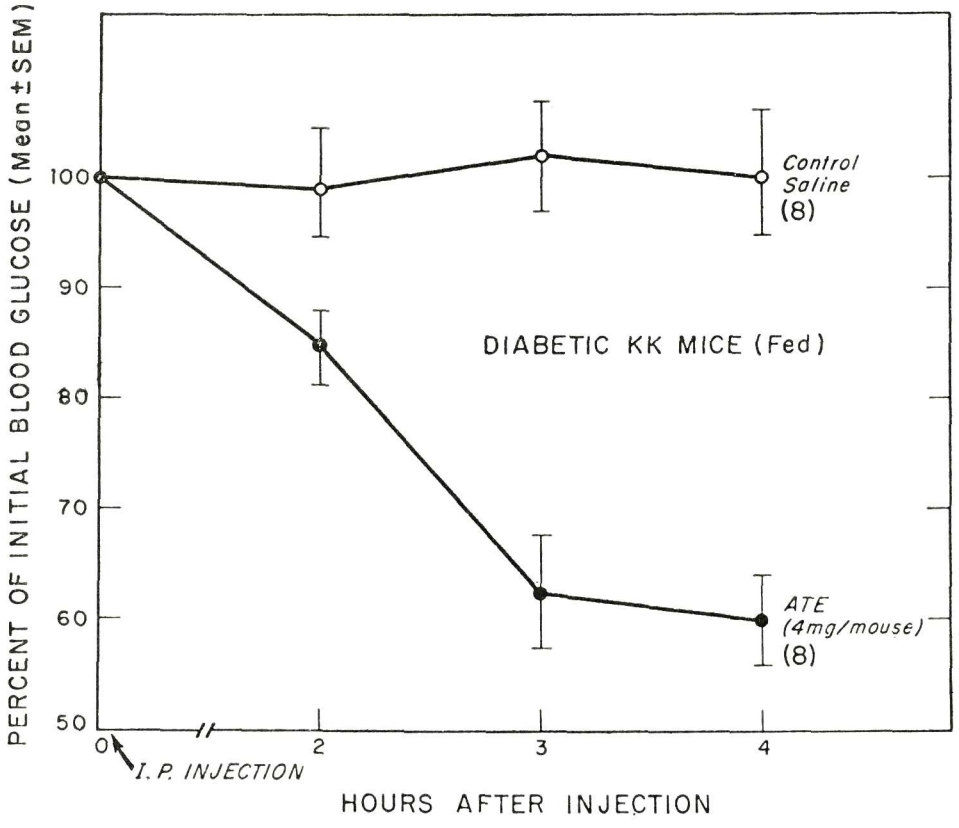

Fig. 4.— Effect of partially purified bovine mesenteric ATE on the blood glucose concentrations of fed, intact, spontaneously diabetic mice of the KK strain. (Initial blood glucose concentration : 128 ± 5.6 mg %).

trations of these animals lasting 24 hours. Blood samples obtained 30 hours after ATE administration (174 and 270-hour samples) exhibited significantly lower blood glucose concentrations than the control animals. Forty-eight hours after the ATE injection the blood glucose returned to the preinjection levels. The blood glucose concentrations of animals injected with control saline were not affected (Figure 5).

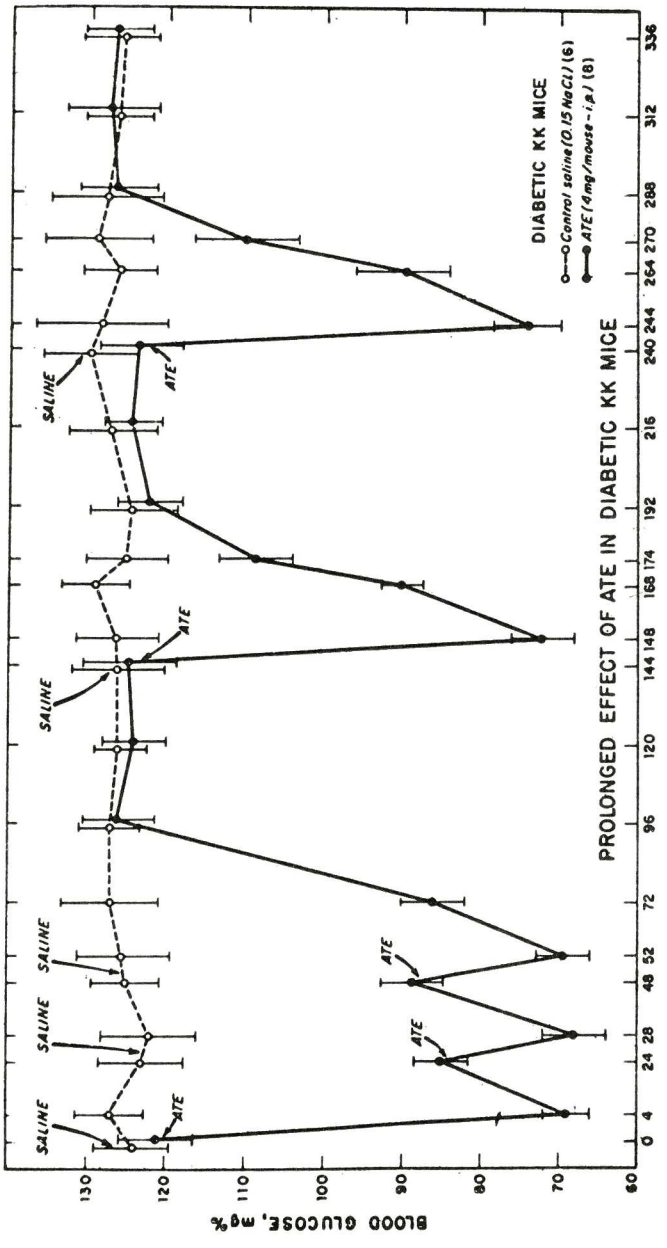

Fig. 5.— Long-term studies on the effect of partially purified bovine mesenteric ATE on the blood glucose concentrations of intact, spontaneously diabetic mice of the KK strain.

The animals were fed ad libitum during the 15-day study. Each animal was injected intraperitoneally, as indicated, with 0.5 ml of 0.15 M NaCl with and without ATE.

As shown in Table I, injection of ATE in adrenalectomized rats stimulated an increase in the rate of incorporation of glucose carbon into the liver glycogen and into the epididymal adipose tissue glycogen and fat of adrenalectomized rats. These effects of ATE are similar to those produced with the administration of bound or crystalline insulin in these animals (**16**). The increase in the incorporation of glucose carbon into the muscle glycogen following ATE administration was not statistically significant.

Insulin immunoassay of blood samples obtained from the adrenalectomized rats two, three and four hours after ATE administration, did not show a significant change in immunoreactive insulin as compared with the levels of immunoreactive insulin of animals injected with control saline solution. Immunoreactive serum insulin levels in both groups of animals were below 10 microunits per ml. However, the bound insulin activity in the blood sera of adrenalectomized rats injected with ATE declined significantly three hours after the injection compared with the activity of bound insulin in the sera of animals injected with control saline (Table II). Similarly, the insulin activity in blood sera obtained from KK mice four hours after ATE administration diminished significantly compared to insulin activity in the sera of animals injected with control saline (Table II). The immunoreactive free insulin levels of pooled sera from KK mice injected with control saline were 33 microunits per ml and that of animals injected with ATE 24 microunits per milliliter.

DISCUSSION

The present studies confirm and extend our earlier finding that ATE can produce hypoglycemia in adrenalectomized rats (**7**). Hypoglycemia in these animals was produced with both homologous rat and heterologous bovine mesenteric or perirenal ATE following intravenous or intraperitoneal administration. Of interest is the effect of ATE in the KK mice. These animals are considered to be diabetic with characteristics similar to those of human diabetes of the maturity-onset type. They have elevated glucose tolerance curves and higher than normal pancreatic (**9**) and blood serum insulin content (**17**). Birnie et al. (**17**) reported that the immunoreactive insulin levels of fed KK mice were 111 μU/ml

T A B L E I.

Effect of bovine mesenteric ATE on the incorporation of glucose carbon into the glycogen of liver, muscle and adipose tissue and into the fat of the adipose tissue of adrenalectomized rats *.

Sample Injected	Liver Glycogen CPM/g tissue + SEM	Muscle Glycogen CPM/g tissue + SEM	Adipose Tissue Fat CPM/g tissue + SEM	Adipose Tissue Glycogen CPM/g tissue + SEM
Control Saline + Glucose-U-C^{14}	171 ± 21	819 ± 228	2587 + 465	104 ± 16
ATE + Glucose-U-C^{14} (4 mg)	412 ± 84 ($P<.02$)	1587 ± 449 (N. S.)	4273 ± 197 ($P<.01$)	179 ± 23 ($P<.05$)

* Each animal received intraperitoneally 1 ml of 0.15 M NaCl with and without ATE containing 2 μC glucose - U - C^{14}. The animals were sacrificed two hours after injection. Six animals were used in each group. The values are means of the average counts per minute incorporated in the glycogen of the two hemidiaphragms, two epididymal adipose tissue pads and two liver pieces and into the fat of two epididymal adipose tissue pads of each animal.

T A B L E II.

Intraperitoneal assay for insulin activity of pooled serum samples collected from diabetic KK mice and adrenalectomized rats following administration of ATE or control saline.

Sample	No. of Rats	Muscle Glycogen CPM/g tissue ± SEM	P	Adipose Tissue Glycogen CPM/g tissue ± SEM	P	Adipose Tissue Fat CPM/g tissue ± SEM	P
Bound insulin from ATE-injected adrenalectomized rats	5	4,005 ± 600	<.01	206 ± 23	<.001	2,200 ± 308	<.01
Bound insulin from control saline-injected adrenalectomized rats	5	7,000 ± 682		480 ± 51		4,056 ± 420	
Sera from ATE-injected KK mice	4	11,923 ± 298	<.05	600 ± 260	<.05	5,070 ± 1,007	<.05
Sera from control saline-injected KK mice	4	20,469 ± 3,262		1,460 ± 219		10,080 ± 1,750	
1,000 microunits crystalline insulin	5	13,460 ± 1,567		2,130 ± 286		7,780 ± 820	

Five ml of 5% HSA containing 0.5 ml of serum from KK mice or 1 ml of serum bound insulin from adrenalectomized rats or 1,000 μu crystalline insulin were injected intraperitoneally into each fed rat along with 2 μC glucose-U-C^{14}. Values are means—above control— of the average counts per minute incorporated into the glycogen of the two hemidiaphragms and into the glycogen and fat of the two epididymal adipose tissue pads of each animal.

vs 35 μU/ml for HaM/ICR controls, and those of fasted (18 hr) KK mice 41 μU/ml vs 14 μU/ml for the controls. Intraperitoneal injections of ATE in KK mice both in our laboratories and at the Smith, Kline and French Research Laboratories produced similar results (Figure 3). The effect of ATE in these animals lasted 24 to 30 hours as shown by the long-term studies presented in Figure 5. These data provide conclusive evidence that at least in this type of inbred diabetic animal ATE is effective, producing a significant and prolonged decline in the blood glucose concentrations.

ATE preparations exhibiting *in vivo* activity have been obtained from rat epididymal and from bovine mesenteric and perirenal adipose tissue. Bovine or rat liver and kidney extracts obtained with techniques identical to those used for the preparation of ATE did not affect the blood glucose concentrations of adrenalectomized rats. Lenti et al (**18**), who confirmed our studies with ATE in adrenalectomized rats, also reported no effect of liver and kidney extracts in these animals.

In vitro studies have shown that ATE can activate bound insulin in the presence of isolated hemidiaphragm (**5 - 6**). It was suggested at that time that ATE may trigger tissue factors which in turn catalyze the activation and utilization of bound insulin (**5**). These *in vitro* observations led to the present studies on the *in vivo* effect of ATE in the adrenalectomized rats and the intact diabetic KK mice. It is possible that the ATE - induced hypoglycemia in these animals is due to the activation of their circulating bound insulin by ATE. The bound insulin concentration in the sera of adrenalectomized rats is estimated between 300 - 400 microunits per ml serum. These animals are sensitive to small amounts of insulin. Intact rats which exhibit higher concentrations of bound insulin (400 - 500 microunits per ml serum) did not respond to ATE administration. However, intact rats were shown to be less sensitive to insulin (**14, 16**). On the other hand, hypophysectomized rats which are also sensitive to insulin responded to ATE administration (unpublished data). Studies presented in Tables I and II are consistent with the hypothesis that ATE may stimulated the activation and utilization of bound insulin. In adrenalectomized rats ATE administration stimulated the incorporation of glucose carbon into the liver and adipose tissue glycogen and into the fat of the adipose tissue of these animals.

These effects are similar to those produced by injection of crystalline or bound insulin. More direct evidence is provided by the fact that the insulin activity in the blood sera of ATE-injected KK mice and adrenalectomized rats is considerably lower compared to insulin activity in the blood sera of animals injected with control saline (Table 11). This may indicate a more rapid utilization of bound insulin by tissues under the stimulation of ATE.

Lenti and Pellegrini recently observed that ATE did not affect the blood glucose concentrations of adrenalectomized-alloxanized rats, which exhibited low concentrations of circulating bound insulin (personal communication). This finding is also consistent with the possibility that the action of ATE may be mediated through the activation of bound insulin. However, other possibilities on the mode of action of ATE cannot be excluded. It is conceivable, for example, that the effects of ATE may be due to an insulin-like substance in these preparations which directly stimulates glucose utilization. This supposition, however, is not supported by *in vitro* studies, which showed that ATE alone does not exert an insulin-like effect on isolated muscle and epididymal adipose tissue. Futhermore, it is difficult to explain the prolonged effect of ATE in KK mice only on the basis of a direct action on glucose utilization. One may anticipate a rather short half-life for the low molecular weight active factor in the ATE preparation.

The molecular weight of the active factor in ATE appears to be below 1,000 as judged by ultrafiltration (**7**) and Sephadex chromatography (unpublished data). ATE is stable in dilute acids (0.2 M formic acid; 0.02 M hydrochloric acid) and heating at 100° C for 5 to 20 minutes did not affect its biologic activity. Ashing, on the other hand, destroyed the activity of ATE. Partially purified ATE preparations retained full biologic activity during a three-year storage at —15° C. These preparations were stored both in the dry and aquous frozen state. Highly purified preparations obtained from bovine mesenteric adipose tissue exerted hypoglycemic effects in adrenalectomized rats at a dose level of 0.2 mg per rat (about 1.6 mg per kg body weight). Studies on the chemical structure of these ATE preparations are now in progress.

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SUMMARY

Partially purified preparations of bovine adipose tissue extracts (ATE) produced a significant decline in the blood glucose concentrations of intact, spontaneously diabetic mice of the KK strain following intraperitoneal administration. These mice are considered to represent an inbred strain of diabetic animals similar to human diabetes of the maturity-onset type. Long-term studies in KK mice carried out for a period of 15 days demonstrated that ATE can produce a prolonged effect in these animals lasting 24 to 30 hours.

ATE preparations obtained fram homologous rat or heterologous bovine adipose tissue produced a significant decline in the blood glucose concentration of adrenalectomized rats following intravenous or intraperitoneal administration. This effect of ATE was accompanied by an

increase in the rate of incorporation of glucose carbon into the glycogen of liver and epididymal adipose tissue and into the fat of the adipose tissue of these animals. The concentration of circulating bound insulin decreased significantly in the blood of adrenalectomized rats injected with ATE as compared with animals injected with control saline. These data are consistent with the hypothesis that the effects of ATE on the blood glucose concentrations of these animals is due to activation by ATE of their circulating inactive bound insulin.

ΠΕΡΙΛΗΨΙΣ

Ἐπὶ μακρὸν χρονικὸν διάστημα ὑπετίθετο ὅτι ὁ σακχαρώδης διαβήτης ὀφείλεται εἰς ἔλλειψιν παγκρεατικῆς ἰνσουλίνης καὶ κατὰ συνέπειαν ἀπεδίδετο εἰς ἀνεπάρκειαν τοῦ παγκρέατος. Πειραματικαὶ μελέται εἰς τὰ ἐργαστήριά μας ὑποδεικνύουν τὴν πιθανότητα ὅτι ὁ διαβήτης δύναται νὰ προκληθῇ ὄχι μόνον ἀπὸ ἔλλειψιν ἰνσουλίνης εἰς τὸ πάγκρεας ἀλλὰ καὶ ἀπὸ ἐξωπαγκρεατικὰς ἀνωμαλίας τῶν βιοχημικῶν μηχανισμῶν οἱ ὁποῖοι ρυθμίζουν τὴν βιολογικὴν δρᾶσιν τῆς ἰνσουλίνης.

Παρετηρήσαμεν ὅτι ἡ ἰνσουλίνη κυκλοφορεῖ εἰς τὸ αἷμα ὑπὸ δύο μορφάς, τὰς ὁποίας ἐκαλέσαμεν ἐλευθέραν καὶ δεσμευμένην ἰνσουλίνην. Ἡ ἐλευθέρα ἰνσουλίνη προέρχεται ἀπὸ τὸ πάγκρεας, εἶναι βιολογικῶς δραστικὴ καὶ ἔχει ἰδιότητας παρομοίας μὲ τὴν παγκρεατικὴν κρυσταλλικὴν ἰνσουλίνην. Ἡ δεσμευμένη ἰνσουλίνη εἶναι βιολογικῶς ἀδρανής, ἔχει μεγαλύτερον μοριακὸν βάρος, διαφόρους ἀνοσολογικὰς ἰδιότητας καὶ βραδυτέραν ἠλεκτροφορητικὴν ταχύτητα ἀπὸ τὴν ἐλευθέραν ἰνσουλίνην. Ὑπεδείξαμεν τὴν πιθανότητα ὅτι ἡ δεσμευμένη ἰνσουλίνη ἀντιπροσωπεύει ἕνα ἀδρανῆ μεταβολίτην τῆς ἐλευθέρας ἰνσουλίνης. Ἡ μετατροπὴ αὕτη λαμβάνει χώραν εἰς ἐξωπαγκρεατικοὺς ἱστοὺς — κυρίως εἰς τὸ ἦπαρ — ὑπὸ ὡρισμένας μεταβολικὰς συνθήκας.

Εἰς μὴ διαβητικοὺς ἡ ἰνσουλίνη εἰς τὸ αἷμα κυκλοφορεῖ ὡς δεσμευμένη, βιολογικῶς ἀδρανής, ἐνόσῳ ἡ πυκνότης τοῦ σακχάρου παραμένει φυσιολογική. Ἡ δεσμευμένη ἰνσουλίνη ἐνεργοποιεῖται παρουσίᾳ ηὐξημένης πυκνότητος σακχάρου τοῦ αἵματος. Ἡ βιολογικὴ δρᾶσις τῆς ἰνσουλίνης φαίνεται ὅτι ρυθμίζεται ἀπὸ τὴν ἰσορροπίαν μεταξὺ δραστικῆς καὶ ἀδρανοῦς ἰνσουλίνης.

Εἰς διαβητικοὺς ἀσθενεῖς εὑρισκομένους εἰς τὰ ἀρχικὰ στάδια, ἡ ἰνσουλίνη κυκλοφορεῖ κατὰ μέγιστον ποσὸν ὡς δεσμευμένη, βιολογικῶς ἀδρανής. Ἡ δεσμευμένη ἰνσουλίνη εἰς τοὺς διαβητικοὺς αὐτοὺς δὲν ἐνεργοποιεῖται, παρὰ τὴν μεγά-

λην πυκνότητα σακχάρου τοῦ αἵματος, ἡ ὁποία θὰ προεκάλει τὴν ἐνεργοποίησιν τῆς ἀδρανοῦς ἰνσουλίνης εἰς μὴ διαβητικούς. Αἱ παρατηρήσεις αὗται ἀποδεικνύουν τὴν παρουσίαν βιοχημικῶν διαταραχῶν εἰς τὸν μηχανισμὸν ρυθμίσεως τῆς βιολογικῆς δράσεως τῆς ἰνσουλίνης εἰς διαβητικοὺς ἀσθενεῖς. Αἱ διαταραχαὶ αὗται ὀφείλονται δηλαδὴ πιθανῶς εἰς ηὐξημένην μετατροπὴν τῆς ἐλευθέρας ἰνσουλίνης εἰς δεσμευμένην εἰς τὸ ἦπαρ, καὶ εἰς βραδεῖαν ἐνεργοποίησίν της ὑπὸ τῶν ἱστῶν τῶν διαβητικῶν ἀσθενῶν. Οὕτως, ἀδρανὴς δεσμευμένη ἰνσουλίνη συγκεντροῦται εἰς μεγάλας πυκνότητας εἰς τὸ αἷμα διαβητικῶν ἀσθενῶν. Ἡ πυκνότης τοῦ σακχάρου ἐπίσης παραμένει ηὐξημένη, καὶ διεγείρει περαιτέρω ἔκκρισιν ἐλευθέρας ἰνσουλίνης ὑπὸ τοῦ παγκρέατος, ἥτις καὶ πάλιν δεσμεύεται ὑπὸ τοῦ ἥπατος. Ἐν καιρῷ προκαλεῖται ἐξάντλησις παγκρεατικῆς ἰνσουλίνης καὶ τὸ ἄτομον πλέον γίνεται διαβητικὸν ἐξαρτώμενον ἀπὸ θεραπείαν ἐξωγενοῦς ἰνσουλίνης.

Αἱ *in vitro* παρατηρήσεις μας ὅτι ἐκχυλίσματα λιπώδους ἱστοῦ (Adipose Tissue Extract (ATE)) δύνανται νὰ ἐνεργοποιήσουν τὴν ἀδρανῆ ἰνσουλίνην παρουσίᾳ μυϊκοῦ ἱστοῦ, ἐπεξετάθησαν καὶ εἰς *in vivo* πειραματικὰς ἐρεύνας. Ἐσκέφθημεν ὅτι ἡ ἐνεργοποίησις τῆς ἀδρανοῦς ἰνσουλίνης ὑπὸ ATE *in vivo* δύναται νὰ προκαλέσῃ ἐλάττωσιν τοῦ ἐπιπέδου σακχάρου αἵματος. Ὡς περιγράφεται εἰς τὴν παροῦσαν ἀνακοίνωσιν, ἐνδοπεριτοναϊκὴ ἢ ἐνδοφλέβιος ἔνεσις τοῦ ATE προκαλεῖ σημαντικὴν πτῶσιν εἰς τὸ ἐπίπεδον τοῦ σακχάρου τοῦ αἵματος πειραματοζώων ὑποβληθέντων εἰς ἐπινεφριδιεκτομήν, καθὼς καὶ εἰς διαβητικοὺς ἐπίμυας πάσχοντας ἐκ κληρονομικοῦ διαβήτου παρομοίου μὲ τὸν ἀνθρώπινον διαβήτην. Ἡ δρᾶσις τοῦ ATE εἰς τὰ διαβητικὰ πειραματόζωα εἶναι μακρά, διαρκοῦσα περὶ τὰς τριάκοντα ὥρας. Τὸ μοριακὸν βάρος τῆς ἐνεργοῦ χημικῆς οὐσίας εἰς τὸ ATE ἡ ὁποία προκαλεῖ τὴν ἐνεργοποίησιν τῆς ἀδρανοῦς ἰνσουλίνης εἶναι μικρότερον τῶν 1,000. Ἡ χημικὴ αὐτὴ οὐσία πιθανῶς ἀποτελεῖ ἓν συνένζυμον, τὸ ὁποῖον διεγείρει ἔνζυμα τῶν ἱστῶν, ἅτινα ἐν συνεχείᾳ μετατρέπουν τὴν ἀδρανῆ ἰνσουλίνην εἰς δραστικήν. Πειραματικαὶ ἔρευναι εἰς τὰ ἐργαστήριά μας ἔχουν σκοπὸν τὴν διευκρίνησιν τοῦ χημικοῦ τύπου τοῦ ATE, τὴν σύνθεσίν του ὡς καὶ τὴν μελέτην τῆς θεραπευτικῆς δράσεως τοῦ ATE εἰς διαβητικοὺς ἀσθενεῖς.