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Influence of Sodium β-Hydroxybutyrate on Glucose and Free Fatty Acid Metabolism in Normal Dogs*

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Summary. The metabolic effects of a 120 min sodium β-hydroxybutyrate infusion (5 mmoles/kg/h) were studied in healthy anaesthetized dogs. — Arterial blood sugar decreased progressively, the fall averaging 25 mg/100 ml from the 80th min onwards. A slight, transient increase in plasma insulin concentration was observed with a mean peak of + 8 μU/ml at the 10 th min of infusion. The disappearance rates of a ^{12}C-glucose load or of a tracer of ^{14}C-glucose were not modified by the sodium β-hydroxybutyrate infusion. Isotope dilution calculation showed that hypoglycaemia was entirely the consequence of a reduction in hepatic glucose output. — Plasma NEFA concentration decreased during the first hour, the fall averaging 180 μ moles/l. Subsequently, despite the maintenance of the infusion of ketone bodies, plasma NEFA returned to basal levels or above in some dogs, while remaining at low levels in others. Turnover studies using a constant infusion of ^{14}C-palmitate, revealed that the decrease in NEFA concentration was the consequence of both an inhibition of NEFA outflow from adipose tissue and an increase in the rate of NEFA uptake by the tissues. — Contrary to data of the literature, our results do not suggest that the prolonged inhibitory effect of sodium β-hydroxybutyrate on hepatic glucose output could be mediated through the minor pancreatic stimulation. This could, however, explain the NEFA changes observed.

L'influence du β-hydroxybutyrate de sodium sur le métabolisme du glucose et des acides gras libres chez le chien normal.

Résumé. Les auteurs étudient l'action d'une perfusion de β-hydroxybutyrate de sodium (5 m moles/kg/h pendant 120 min) sur les métabolismes glucidique et lipidique du chien normal anesthésié. — La glycémie artérielle décroît progressivement, la chute atteignant 25 mg/100 ml à partir de la 80e min. L'insulinémie s'accroît légèrement (+ 8 μU/ml) à la 10ème min et retourne à sa valeur basale dès la 30ème min de la perfusion. La vitesse de disparition d'une surcharge i. v. en glucose-^{12}C ou en glucose-^{14}C n'est pas modifiée par l'administration des corps cétoniques. Le calcul de dilution isotopique montre que l'hypoglycémie est entièrement secondaire à une réduction du débit glucosé du foie. — La concentration plasmatique moyenne des NEFA diminue de 180 μM/l pendant la 1ère heure de la perfusion. Ultérieurement, malgré le maintien de celle-ci, la lipacidémie s'élève chez certains animaux jusqu'à des taux dépassant les valeurs basales, tandis que l'abaissement persiste chez d'autres. La vitesse du «turnover» des NEFA, mesurée par la technique de perfusion continue de palmitate-1-^{14}C montre que la chute des NEFA résulte des effets conjugués d'une baisse de leur production par le tissu adipeux et d'une augmentation de leur vitesse de captation par les tissus consommateurs. — Ces résultats sont en opposition avec certaines données de la littérature parce qu'ils excluent que la chute de la glycémie soit secondaire à la faible stimulation pancréatique observée. Celle-ci peut cependant rendre compte des modifications transitoires de la lipacidémie.

Der Einfluß von Natrium β-Hydroxybutyrat auf den Stoffwechsel der Glucose und der freien Fettsäuren beim normalen Hund.

Zusammenfassung. An anästhesierten Hunden untersuchten wir die Stoffwechselwirkungen einer Infusion von β-Hydroxybutyrat (5 m/moles/kg/Std) über 2 Stunden. — Wir beobachteten eine zunehmende Blutzuckersenkung, die nach der achtzigsten Minute i.D. 25 mg% betrug. Ferner trat ein leichter, vorübergehender Anstieg der Plasmainsulin-Konzentration von i. D. 8 Mikroeinheiten/ml 10 min nach Beginn der Infusion ein. Die Schwundraten von ^{12}C-Glucose oder ^{14}C-Glucosegaben änderten sich unter der Infusion nicht. Berechnungen der Isotopen-Verdünnung zeigten, daß die Blutzuckersenkung lediglich auf einer verminderten Glucosefreisetzung aus der Leber beruht. — Die Plasmakonzentration der freien Fettsäuren sank während der ersten Stunde um durchschnittlich 180 Mikromol/l. Danach kehrte sie bei einigen Hunden trotz Fortsetzung der Ketokörper-Infusion auf den Ausgangswert oder sogar höhere Konzentrationen zurück, während sie bei anderen Tieren erniedrigt blieb. Umsatzstudien mit Hilfe einer Dauerinfusion von ^{14}C-Palmitat ergaben, daß der Abfall der FFS-Konzentration durch eine Verringerung der FFS-Abgabe des Fettgewebes und eine vermehrte FFS-Aufnahme in anderen Geweben bedingt war. Im Gegensatz zu den Angaben der Literatur weisen unsere Ergebnisse nicht darauf hin, daß die anhaltende Hemmung der Glucose-Abgabe aus der Leber durch Natrium β-Hydroxybutyrat über die geringfügige Stimulierung des Pankreas zu erklären ist. Doch könnte diese die aufgetretenen Änderungen der FFS-Konzentrationen bewirken.

Key-words: Sodium β-hydroxybutyrate infusion in dogs, effects on ^{12}C- and ^{14}C-glucose, insulin, NEFA, ^{14}C-palmitate

Introduction

Although the mechanisms involved in hepatic ketogenesis are incompletely understood, it is well established that the production of ketone bodies is increased when the mobilization of lipids is enhanced, as it is during carbohydrate deprivation.

Conversely, recent work suggests that ketone bodies might interfere with carbohydrate and lipid metabolism. *In vitro,* ketone bodies added to the incubation medium inhibit glucose uptake and oxidation by muscle [28, 14, 24] and decrease the lipolysis of adipose tissue [5]. Madison and coworkers [21, 19] have shown that ketone bodies, when administered *in*

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vivo, provoke metabolic disturbances chiefly characterized by a stimulation of insulin release and a fall in the plasma concentrations of glucose and NEFA.

The purpose of the present work is to study the kinetics of glucose and NEFA modification during sodium β-hydroxybutyrate (Na β-OH-B) infusions in dogs by means of isotopic methods using glucose -1-^{14}C and palmitate -1-^{14}C.

Materials and Methods

The studies were performed on adult mongrel dogs weighing 17—30 kg, fasted 18—20 h. Anaesthesia was induced with Pento-barbital (15 mg/kg) and maintained by further smaller doses according to requirements. The different experimental protocols used are defined in the figures. The measured parameters were the following: blood sugar concentration, plasma concentration of NEFA, insulin and ketone bodies, "K value" of the intravenous glucose tolerance test and turnover rates of glucose and NEFA estimated by isotopic dilution methods. Blood samples for all chemical and isotopic determinations were drawn from the femoral artery via an indwelling Cournand needle. All the substances administered to the dogs were injected or infused through indwelling polyethylene catheters inserted in the saphenous veins. After a control period, Na DL-β-OH-B was infused as an aqueous solution, the animals receiving 5 mmole/kg/h under a constant flow of 73.2 ml/h. The pH of the Na β-OH-B solution was alkaline (pH 8) and was not neutralized before injection, since it has been proven that alkalinity has no influence on the metabolic effect of the substance [21].

Analytical methods

Blood sugar concentration was determined on heparinized blood samples containing fluoride, by a modification of HOFFMAN's method [17], adapted to the Auto-Analyser.

The other chemical determinations were performed on plasma samples, which were obtained from heparinized blood immediately centrifuged under refrigeration, and kept frozen at — 20 °C until the day of the analysis. *NEFA concentration* was estimated following DOLE's procedure [10], *total ketone bodies* following a modification [18] of MICHAEL's method [22] and *plasma insulin* according to the method of MORGAN et al. [23].

The intravenous glucose tolerance test was performed following the technique described by CONARD et al. [8, 7]: rapid i.v. glucose loading (0.5 g/kg body weight in a 50% solution); calculation of the glucose disappearance rate by graphical procedure from the 7th till the 31st min on a semi-logarithmic scale (log. of blood sugar concentration as ordinate, time as abscissa).

Glucose turnover rate: sixty μC of glucose-1-^{14}C (2 mC/mM) was rapidly injected by the venous route and allowed to equilibrate in the extracellular fluid for 40 min. From that time onwards the ^{14}C-glucose content of the whole blood was followed by frequent sampling. The blood ^{14}C-glucose was isolated as $^{14}CO_2$ by a fermentation method using *leuconostoc mesenteroides* [4, 9]. The $^{14}CO_2$ produced was trapped by Hyamine and directly counted by liquid scintillation in a P.P.O. (4 g/l) — P.O.P.O.P. (100 mg/l) — toluene medium. The recovery of $^{14}CO_2$ from glucose-1-^{14}C provided by this method varied from 85 to 92%. The counting efficiency averaged 72%. Results were corrected for counting efficiency but not for incomplete recovery of radioactivity. The ^{14}C-glucose fractional disappearance rate was determined graphically on a semi-logarithmic graph (time as abscissa, ^{14}C-glucose content of the blood as logarithmic ordinate). The influx-efflux rate of glucose was only calculated during the steady state periods using the formula:

$$F = K^* C$$

where F is the influx-efflux rate expressed in mg/100 ml/min; K^* the fractional disappearance rate in % per min; C, the blood glucose concentration (mg/100 ml) averaged for each steady state period.

NEFA turnover rate

Preparation of labelled palmitate for injection: 20 mg of unlabelled palmitic acid was added as carrier to 500 μC of palmitic acid-1-^{14}C (36.6 mC/m/mole) in benzene solution. The solution was evaporated to dryness under a flow of nitrogen and redissolved in 1 ml ethanol. After addition of 5 ml of 0.02 N NaOH, the alcohol was evaporated at 100 °C and the aqueous palmitate-^{14}C soap solution was diluted to a final volume of 100 ml with a 10% solution of crystalline bovine albumin in saline. The preparation was divided into 10 aliquots containing 50 μC each and stored at — 20 °C until use. On the day of the experiment 50 μC of the ^{14}C-palmitate albumin complex was diluted to 100 ml with saline, and infused intravenously throughout the experiment at a constant flow of 37 ml/h regardless the weight of the dog.

Estimation of plasma NEFA and ^{14}C-palmitate concentrations: from each plasma sample, duplicate 1 ml aliquots were extracted following DOLE's technique. One of them was used for measurements of unlabelled NEFA; the other one for estimation of radioactive palmitate, according to the following procedure adapted from WINCKLER et al. [29]: the heptane phase was removed and pooled with another 3 ml heptane extract of the underlying phase; 2 ml of 0.05 N NaOH in 30% ethanol solution was added; after mixing and centrifuging, the upper heptane phase containing the neutral plasma lipids was removed and discarded, and the lower alcoholic phase washed with an additional 3 ml of heptane; the alcoholic phase was then acidified with 0.5 ml of 0.5 N H_2SO_4 and extracted twice with 3 ml portions of heptane; these heptane extracts were pooled in a scintillation-counting vial together with 6 ml of toluene containing P.O.P.O.P (200 mg/l) and P.P.O. (8 g/l) and counted with an efficiency of about 85%. When palmitic acid-1-^{14}C was complexed with albumin and then carried through the analytical

procedure, 86—97% of the radioactivity was recovered. Results were corrected for counting efficiency but not for incomplete recovery of radioactivity.

Calculations: when palmitate-1-^{14}C was infused at a constant rate, a plateau of ^{14}C-palmitate concentration was reached approximately 10 min after starting the infusion. Fractional turnover rates and total NEFA influx or efflux have been calculated by the following formula [16] during the steady state periods (stable levels of both ^{14}C-palmitate and unlabelled NEFA):

$$K = \frac{i}{Q}$$

where K is the fractional turnover rate (min^{-1}); i the radioactivity infused (d.p.m/min); Q the total radioactivity in plasma NEFA (d.p.m)

$$F = \frac{i}{\text{S.A.}}$$

where F is the total influx or efflux of nonlabelled NEFA (μmoles/min); S.A., the plasma NEFA specific activity (d.p.m./μmole).

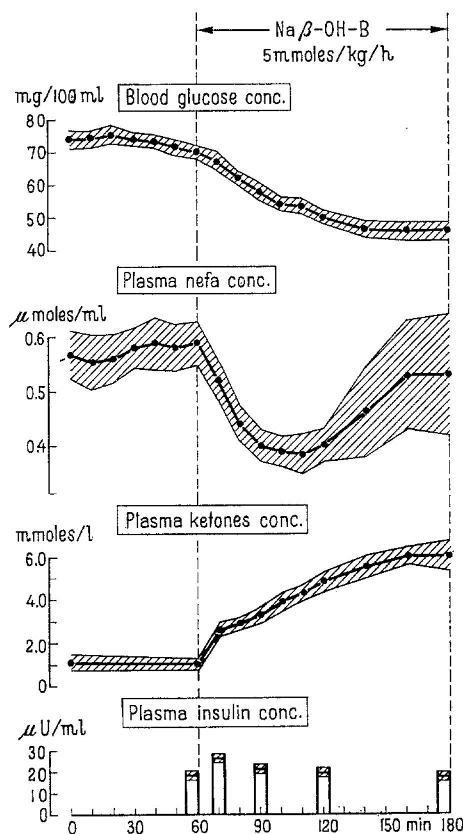

Fig. 1. Influence of a constant Na β-OH-B infusion on blood glucose concentration and plasma concentration of NEFA, total ketone bodies and insulin. Mean results for 9 dogs ± S.E.M.

Results

The mean values of blood glucose concentration and of plasma concentration of NEFA, total ketones and insulin for the control period and the Na β-OH-B infusion period are illustrated in Fig. 1.

The modifications induced by the ketone bodies were the following. 1. A decrease in blood sugar which plateaued for the last 40 min of the infusion, representing a mean fall of 25 mg/100 ml. 2. A plasma NEFA decrease averaging 180 μmoles/l at the end of the first hour of infusion. After this initial decline, the behavior of the plasma NEFA was variable from dog to dog. In some of them, there occured a secondary rise bringing the NEFA levels up to basal values or above, though in others plasma NEFA remained at low levels until the end of the infusion period. 3. A progressive rise in ketonemia reaching the mean value of 6 mmoles/l at the end of the infusion. 4. A small increase in plasma insulin concentration, observed in each experiment and statistically highly significant. This effect was transient reaching its peak at the 10th min of infusion (+ 8 μU/ml), and was not detectable after the 30th min.

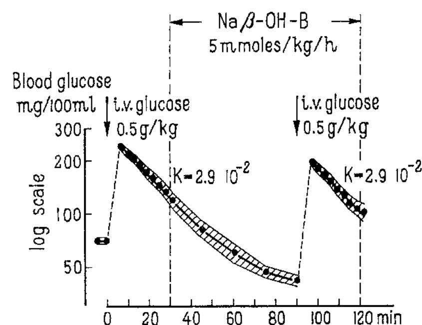

Fig. 2. Effect of a constant Na β-OH-B infusion on the intravenous glucose tolerance test in 6 dogs. Mean results ± S.E.M.

Fig. 2 shows the mean values of blood sugar concentration observed during two successive intravenous glucose tolerance tests. The "K value" averaged 2.9% per min in the basal period and was unaffected by the Na β-OH-B infusion, despite its major hypoglycaemic action.

The influence of Na β-OH-B on glucose turnover is illustrated in Fig. 3. The amounts of radioglucose injected not being proportional to the body weight of the animals, results are expressed in percentage of the blood activities (d.p.m/ml) extrapolated to time zero. During the control period and the 2h infusion of Na β-OH-B, the decrease in glucose-1-^{14}C concentration was linear and constant, corresponding to a fractional turnover rate of 1.1% per min. Liver glucose output, estimated for the two steady state periods (control period and the last 40 min of the Na β-OH-B infusion), showed a significant decrease ($p < 0.001$) from 0.712 mg/100 ml/min to 0.447 mg/100 ml/min under the influence of the administration of ketone bodies. These data reveal that the hypoglycaemic action of Na β-OH-B is due only to a reduction in liver glucose output and not to any modification of the overall glucose utilization rate.

Data pertaining to the turnover of NEFA are presented in fig. 4. Ten min after starting the constant infusion of radioactivity, the plasma concentration of palmitate 1-^{14}C reached a plateau. From this moment, little variation in the concentration and specific activity of NEFA occured during the control period. Na β-OH-B infusion induced a decrease in both labelled and unlabelled plasma NEFA content and a rise in plasma specific activity. Thus, two distinct mechanisms are involved in the lowering of plasma NEFA by ketone bodies: 1. an increase in the rate of NEFA uptake by the tissues; 2. an inhibition of NEFA influx into the blood from adipose tissue.

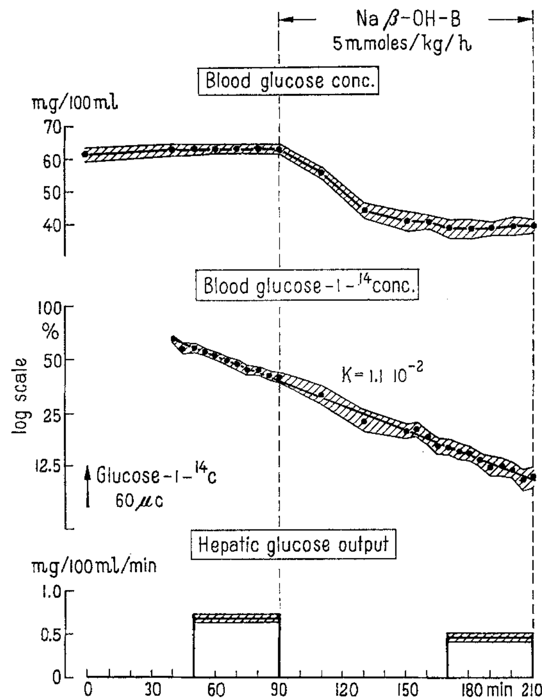

Fig. 3. Influence of a Na β-OH-B constant infusion on blood glucose concentration, ^{14}C-glucose fractional disappearance rate and estimated liver glucose output. The mean blood ^{14}C-glucose concentrations are expressed in percentage of the blood activities (d.p.m/ml) extrapolated to time zero. Mean results for 8 dogs ± S.E.M.

The individual data of these experiments are summarized in Table 1. The fractional turnover rates and the influx or efflux rate of nonlabelled NEFA are also given; they are estimated for the last 20 min of both the control period and the ketone infusion period, which corresponded to steady states. The fractional turnover rates were calculated by assuming that the plasma volume represented 5% of the body weight and remained constant throughout the experiment. For the control period, the fractional turnover rate averaged 35% per min, and was systematically increased to give a mean of 42.8% per min, the rise being statistically

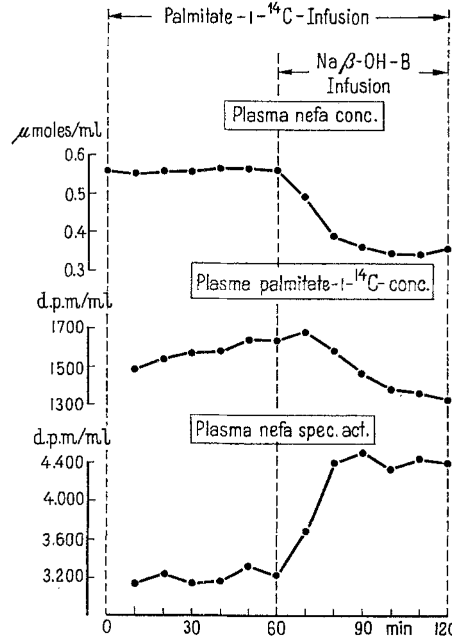

Fig. 4. Influence of a Na β-OH-B infusion on plasma NEFA concentration, palmitate-1-^{14}C concentration and NEFA specific activity during a constant infusion of palmitate-1-^{14}C. Mean results for 9 dogs

Table 1. *Influence of Na β-OH-B on NEFA turnover*

Experiment No	Body Weight (kg)	Influx rate of ^{14}C-palmitate (d.p.m./min)[1]	Plasma Palmitate ^{14}C conc (d.p.m./ml)		Plasma NEFA Spec. Act (d.p.m./μmole)		Fractional turnover rate of NEFA (%/min)		Nonlabelled NEFA influx (μmole/min)	
			Control[2]	Na β-OH-B[3]	Control[2]	Na β-OH-B[3]	Control	Na β-OH-B[3]	Control[2]	Na β-OH-B[3]
16	23	634440	1239	818	1807	2803	44.5	67.4	351	226
17	21	605200	1688	1395	2666	3439	34.2	41.3	227	176
18	17	659600	2008	1775	3298	6117	38.6	43.7	200	108
19	28	632400	1343	1063	2187	3352	33.6	42.5	289	189
20	28	622200	1128	922	1879	2092	39.4	48.2	331	297
22	29	584800	1026	981	1427	1746	39.3	41.1	410	335
23	25	625600	1201	1011	3660	3428	41.7	49.5	171	182
30	19	632400	2966	2654	7990	9417	22.4	25.1	79	67
31	30	625600	1934	1565	4748	7020	21.6	26.7	132	89
Mean							35.0	42.8	243	185

[1] Calculated from the radioactivity recovered by the described procedure (see text).
[2] Mean of 3 values obtained during the last 20 min of the control period (steady state).
[3] Mean of 3 values obtained during the last 20 min of the Na β-OH-B infusion period (steady state).

highly significant ($0.001 < p < 0.01$). Simultaneously, the mean rate of influx or efflux of unlabelled NEFA decreased from 243 μmoles/min to 185 μmoles/min. The fall occured in 8 of the 9 dogs studied and was also highly significant ($0.001 < p < 0.01$).

Six control experiments were performed using infusion of sodium chloride (5 mmoles/kg/h) instead of Na β-OH-B (5 mmoles/kg/h). In these experiments no significant modifications in the concentrations of either blood glucose or plasma labelled and unlabelled NEFA were observed.

Discussion

The present studies afford quantitative data regarding glucose and NEFA metabolism during infusions of Na β-OH-B.

A. Glucose metabolism

In our experimental conditions, the hypoglycaemic action of the infused ketone bodies does not seem related to the observed β-cell stimulation. Indeed the increase in insulin concentration of peripheral blood was small and transient, whereas hypoglycaemia was present throughout the infusion. Moreover, if the hypoglycaemia was mediated through an insulinic effect, an increase in the rate constant of glucose utilization should be observed; such an increase was not found whether the rate constant was measured by the i.v. glucose tolerance test and or by the isotopic method. Lastly our data show that the hypoglycaemia was brought about by a reduction in the glucose output of the liver. It has been proved that during insulinic hypoglycaemia in the normal anaesthetized dog, the inhibitory influence of insulin on the liver glucose output is always overwhelmed by the stimulating effect of peripheral hypoglycaemia [13, 27]. Only when hypoglycaemia is reduced by glucose infusion does the hepatic action of insulin become evident in the normal dog [20, 26]. Since hypoglycaemia cannot be explained by an insulinic effect, the hypothesis of a direct inhibitory action of the ketone bodies upon liver glucose output must be considered.

The finding that Na β-OH-B does not inhibit the rate constant of peripheral glucose uptake by the whole tissues is in contrast with the effects observed *in vitro* on muscle, i.e. with isolated diaphragm or perfused heart.

Our results are in many ways opposed to those obtained by MADISON et al. [21, 19], who noticed that the Na β-OH-B infusion produces an important and prolonged pancreatic stimulation, which they hold responsible for the observed decrease in liver glucose output. They also suggested a ketone-induced inhibition of insulin action on glucose uptake, since in their experiments peripheral glucose utilization was altered despite higher blood levels of insulin.

The discrepancies existing between MADISON'S group conclusions and ours are probably due to differences in the experimental conditions: 1. these authors measured the insulin concentration in the pancreatic venous plasma and gave a heavier load of ketone bodies; 2. they performed their experiments on dogs with porto-caval shunts, and their assumption regarding the action of insulin on liver glucose output obviously cannot be extended to the normal anaesthetized dog, as mentionned above; and 3. they measured the peripheral glucose utilization with an indirect, non-isotopic method, based upon the comparison between the liver balance and the size of the glucose pool, whereas our measurements were direct estimations of glucose utilization rate by the whole tissues.

Still more elaborated are the conclusions of FELTS et al. [12]. Using sodium acetoacetate instead of Na β-OH-B, they showed that the peripheral glucose utilization was enhanced when ketone bodies were infused alone, whereas acetoacetate lowered glucose assimilation rate when administered to dogs rendered hyperglycaemic by a constant glucose infusion.

Infusing acetoacetate at the rate of 1.52 mmoles/kg/30 min, FAJANS et al. [11] observed no modifications in peripheral insulin concentration. These data are in fair accordance with our results, although a precise comparison is not possible, due to differences in the chemical compound used, the doses injected and the species used.

B. NEFA Metabolism

In the discussion of the action of Na β-OH-B on NEFA metabolism, it is necessary to consider successively the behaviour of plasma NEFA during the first and the second hour of in fusion of ketone bodies.

During the first hour of infusion, a systematic drop of NEFA plasma concentration was observed. The use of an isotopic method made it possible to show that this drop was the consequence of both an increase in NEFA uptake by the tissues and a decrease of NEFA production by adipose tissue. The small recorded rise in plasma insulin concentration can provide an explanation for the observed NEFA modifications for the following reasons: 1. small doses of insulin having a negligible influence *in vivo* upon carbohydrate metabolism may exhibit a clear-cut action on NEFA metabolism [30, 1]; and 2. insulin may promote a reduction in NEFA production by adipose tissue and an increase of their uptake, in particular at the hepatic level [25, 6].

Our results pertaining to the action of ketone bodies on plasma NEFA are consistent with the findings of MEBANE et al. [21]. Nevertheless, a direct action of Na β-OH-B on adipose tissue cannot be excluded. Indeed, BJORNTORP has recently provided evidence for an inhibitory effect of Na β-OH-B on rate of lipolysis of adipose tissue *in vitro* [5]. However the action of Na β-OH-B on adipose tissue seems to be a complex one, since HANSON observed an increase in NEFA release in the presence of Na β-OH-B [15].

During the second hour of infusion, no uniform response of the plasma NEFA was recorded: they remained at low levels in some animals and strikingly rose in others. This effect could be interpreted either as

the consequence of the return of the blood insulin to basal level, or as resulting from the adaptative reactions to hypoglycaemia.

When one considers the metabolic effects of Na β-OH-B as a whole, one could be surprised by their similarity with those produced by sulfonylurea in dogs. Like ketone bodies, sulfonylurea provokes a transient increase in insulin secretion but their prolonged hypoglycaemic action is caused by an inhibition of the liver glucose output [3]. Their action on NEFA concentration is also biphasic, with an initial fall induced by the insulin secretion, and a secondary rise that is probably the consequence of the prolonged non-insulinic hypoglycaemia and hence to the glucose deficiency at the peripheral level [2].

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