toxicity appears when the ingestion ratio is greater than 0.1. This hypothesis was tested directly as follows. A group of 12 male Sprague-Dawley rats housed 2 to a cage was fed a K-deficient diet (Nutritional Biochemicals) and given drinking water which contained 10 mmoles/l each of KCl and RbCl. Since each rat consumed an average of 14 g of food daily and 30 ml of water, the K intake was equivalent to at least 0.085% of the diet. After 25 days 5 rats were dead. Weight gain was minimal with all rats. Ten of the 12 rats had 1 or more audiogenic seizures (induced by allowing compressed air to escape through a valve for 30 sec). At that time the surviving rats were returned to a normal diet (containing 0.89% K dry weight). Weight gain was apparent on the 7th day at which time susceptibility to audiogenic seizures disappeared. On the 15th day they were returned to the K-deficient diet. Within 2 weeks all were again susceptible to audiogenic seizures and an additional 3 rats had died. This experiment was repeated on a group of 32 rats (male and female), with the exception that they were not subjected to the compressed air stimulus. At the end of the 4th week 19 of the rats were dead. Thus it was not the Rb level per se, but its ratio to K intake, that determined toxicity.

Since the dietary requirement for K can be met in part by substituting Rb[4,7,13], the appearance of toxicity when Rb in the diet is equivalent to more than 10% of the K indicates that accumulation of Rb by tissues and organs has proceeded to a level at which another effect of Rb assumes quantitative importance. Below that level Rb either serves entirely as a substitute for K or its actions are compatible with normal cell function; above that level either its own physiological actions perturb cell function (in the same sense that an excess of K would be toxic), or new wholly pathological actions become apparent.

It is clear from the data that continued ingestion of Rb is compatible with survival and health. Although Rb was not demonstrably toxic, it was not without effect on the central nervous system. There was a general increase in excitability, as suggested by maternal attacks on the young and the ease with which the animals were startled.

Finally when the animals were made toxic by restricting the K intake to levels equimolar with Rb intake, it was observed that the resultant failure to gain weight and susceptibility to audiogenic seizures could be reversed by increasing the K intake without diminishing the Rb intake. Thus in rats Rb toxicity appears to be reversible[15].

Résumé. En vue de l'emploi anticipé du RbCl en médecine, la présente étude établit les conditions d'administration chronique et sans toxicité chez le rat. L'administration chronique étendue à 3 générations de rats Sprague-Dawley ne révèle aucun effet sur la fertilité, la gestation, le développement ou la longévité.

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Influence of Dihydroergotamine on the Lipolytic System of Isolated Dog Fat Cells

It has been shown by several workers that dihydroergotamine (DHE) antagonizes catecholamine-induced lipolysis and, at higher concentration also ACTH-induced lipolysis[1-4] in isolated rat fat cells. FAIN[2], HOTTA et al.[5] and our experiments showed that DHE was moderately lipolytic by itself in vitro. We found a maximal lipolytic activity with about $10^{-5}M$ DHE (unpublished results) in rat fat cells suspensions.

In vivo, we found that DHE does not stimulate the lipolysis in fasted rats. It was also shown[6] that DHE completely blocks epinephrine-induced increase of plasma free fatty acids.

In the fasted dog, SCRIABINE et al.[7] showed that DHE in doses of 0,125 and 0,5 mg/kg i.v. increases plasma concentration of FFA. These findings were confirmed by SIREK et al.[8] and in our laboratory. In their work, SCRIABINE et al.[7] concluded that DHE may stimulate adrenergic receptors of the adipose tissue, or activate lipolytic mechanisms beyond adrenergic receptors.

By in vitro experiments, we attempted to explain the mode of action of DHE on the isolated dog fat cells.

Methods. Preparation of isolated fat cells. Fasted mongrel dogs (5–12 kg) both sexes, anaesthetized with sodium pentobarbital (30 mg/kg) were used as donors of subcutaneous adipose tissue. The fat cells were isolated by the method of RODBELL[9], modified so that the incubation medium used for the digestion of the tissues did not contain glucose. After isolation, the fat cells were suspended in a Krebs-Ringer phosphate (KRP) solution, pH 7.4 with 4% albumin. One-ml portions of the suspension were given into 25-ml plastic vials containing 1 ml of KRP with the drugs to be tested. After a 2 h incubation at 37 °C with air, the incubation medium was analyzed for glycerol content using the method of LAURELL and TIBBLING[10]. The results are expressed as μmoles of glycerol per mmole of triglycerides in the cell suspension.

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Preparation of fat cells 'ghosts'. Plasma membrane sacs (ghosts) were prepared by lysing isolated fat cells as described by RODBELL[11].

Phosphodiesterase assay. The phosphodiesterase activity of adipose tissue homogenates was measured by the method of Ho et al.[12] with some modifications: The reaction was stopped by adding 300 µl of cold water and transferring the assay tubes into boiling water for 3 min. After a 10 min centrifugation at 2000*g*, 100 µl of the supernatant was taken and the radioactive cyclic AMP not destroyed by phosphodiesterase was separated by adding 0,2 ml each of $ZnSO_4$ (0.25*M*) and $Ba(OH)_2$ (0.25*M*). The samples were mixed and centrifuged. As we could see, a single precipitation by $BaSO_4$ was enough to eliminate the impurities. The radioactivity was counted in the supernatant and the results expressed as nmoles cyclic AMP per mg protein. The protein content of the adipose tissue homogenate was determined by the method of LOWRY et al.[13].

Adenyl-cyclase assay. Adenyl-cyclase activity was studied in fat cell 'ghosts'. The method used was that described by RODBELL[11], using the ATP-regenerating system. Adenosine-8-^{14}C-5'-triphosphate was used and 0.1% albumin was added to the incubation medium. After centrifugation of the boiled reaction mixture, 0.5 ml of the supernatant was transferred to a column for chromatography and the cyclic AMP isolated as described by WEISS and COSTA[14]. As for the phosphodiesterase assay, the results are expressed as nmoles cyclic AMP per mg protein and per 10 min.

Results. The results of one particular experiment are presented but they are representative of at least 3 experiments, individual results being the mean of triplicate assays.

Effect of DHE on basal lipolysis. As shown in Figure 1, the lipolytic effect of DHE, which had been observed in vivo, can be reproduced in the isolated dog fat cells. At about $3.2\times10^{-7}M$, DHE produces maximal lipolysis. Higher concentrations give a reduced response. From the positions of the maxima of the 2 curves it is tentatively concluded that DHE has a higher affinity for the lipolytic system than norepinephrine (NE).

Effect of various concentrations of DHE on the NE-induced lipolysis. With isolated dog fat cells, noradrenolytic action of DHE is only seen in high concentrations ($10^{-4}M$) as Figure 2 shows. Figure 3 shows the effect of

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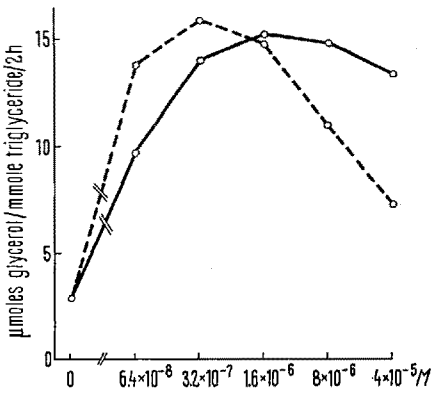

Fig. 1. Lipolytic effect of NE (O—O) or DHE (O---O) in isolated dog fat cells.

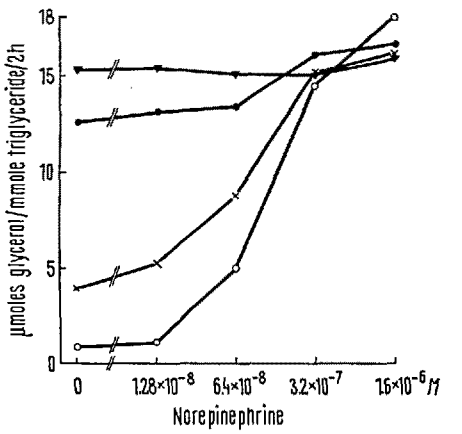

Fig. 3. Effect of various concentrations of DHE on NE-induced lipolysis in isolated dog fat cells. ×—×, NE + DHE $4\times10^{-8}M$; ●—●, NE + DHE $2\times10^{-7}M$; ▼—▼, NE + DHE $1\times10^{-6}M$; O—O, NE alone.

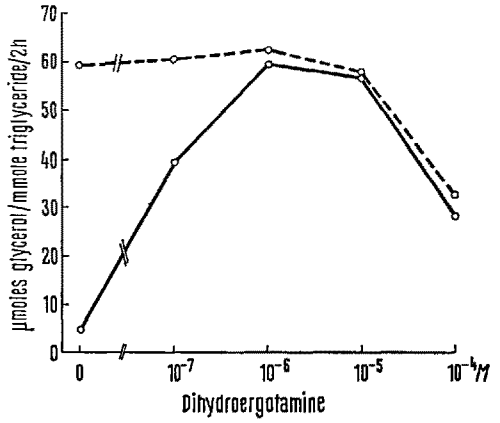

Fig. 2. Effect of various concentrations of DHE on NE-induced lipolysis (NE $1.6\times10^{-6}M$) in isolated dog fat cells. DHE alone, O—O; DHE + NE, O---O.

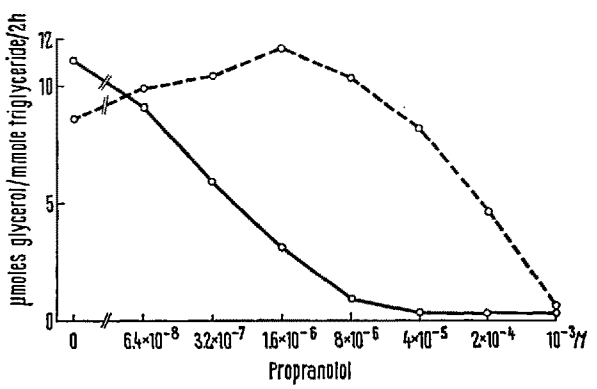

Fig. 4. Effect of propranolol on NE-induced lipolysis (NE $8\times10^{-6}M$) O—O or DHE-induced lipolysis (DHE $8\times10^{-6}M$) O---O in isolated dog fat cells.

3 different concentrations of DHE on the dose-response curve of NE. By adding submaximal concentrations of DHE to various concentrations of NE, additivity of the lipolytic effect of NE and DHE was observed. However, the maximal lipolysis obtainable with NE alone could not be exceeded by the addition of DHE. This effect is due to the saturation of the lipase activity.

Effect of propranolol on the NE- or DHE-induced lipolysis. The antagonistic action of the β-blocker propranolol was tested on DHE-induced lipolysis in comparison with its effect on NE-induced lipolysis. The results are summarized in Figure 4, which shows that the concentration of propranolol required to inhibit the glycerol release due to DHE is 100 times higher than that required to inhibit the release due to NE. A slight potentiation of the lipolytic effect of $8 \times 10^{-6} M$ DHE by propranolol ($1.6 \times 10^{-6} M$ to $8 \times 10^{-6} M$) was observed.

Effect of insulin on the NE- or DHE-induced lipolysis. It is known that insulin inhibits non-competitively the cyclic AMP production stimulated by catecholamines and that consequently triglyceride mobilization decreases[15–17]. It was interesting to see if insulin would similarly inhibit the DHE-induced lipolysis. The results summarized in Table I show that insulin inhibited also the lipolysis stimulated by DHE. The results reported in Figures 1–4 and in Table I suggest that DHE acts before the formation of the active lipase by stimulating adenylcyclase and/or by inhibiting phosphodiesterase.

Effect of DHE on phosphodiesterase activity in adipose tissue homogenate. The effects of DHE and theophylline, a known inhibitor of phosphodiesterase, were compared. A complete inhibition of the cyclic AMP degradation was obtained with theophylline $10^{-2} M$, but cyclic AMP disappearance from the medium was the same for DHE $10^{-5} M$ as for the controls. Thus, DHE in a concentration of high lipolytic activity had no effect on phosphodiesterase activity.

Table I. Effect of insulin on DHE-induced lipolysis in isolated dog fat cells

Concentration of DHE (M)	Glycerol release (μmoles glycerol/mmole triglyceride/2 h) Without insulin	With insulin (1 mU/ml)
0	1.89	–
6.4×10^{-8}	12.96	5.03
3.2×10^{-7}	28.05	13.46
1.6×10^{-6}	24.15	19.25
8×10^{-6}	21.13	19.87
4×10^{-5}	15.72	13.58

Table II. Effect of DHE, compared with NE, on adenylcyclase activity in dog fat cells 'ghosts'

Additions to incubation medium (M)	Cyclic-AMP (nmoles/mg protein/10 min)
None	2.01
Norepinephrine 10^{-5}	2.66
Norepinephrine 10^{-4}	3.64
Norepinephrine 10^{-3}	4.33
DHE 10^{-6}	2.26
DHE 10^{-5}	2.80
DHE 10^{-4}	2.74

Effect of DHE on adenyl-cyclase activity in fat cells 'ghosts'. The effects of DHE and NE on adenyl-cyclase activity were compared. DHE ($10^{-5} M$) showed a significant increase (39%) in the production of cyclic-AMP, as presented in Table II. It was observed that the intrinsic activity of DHE is lower than that of NE but the increase in adenyl-cyclase activity obtained with $10^{-5} M$ DHE is sufficient to explain at least part of the stimulation of the lipolytic processes observed.

Discussion. Up to now, most of the biochemical research concerning the mechanisms of action of DHE deals with its antilipolytic effect. It is well established that DHE inhibits the activation of hormone-sensitive lipase by NE, ACTH and theophylline at biochemical site(s) at some stage subsequent to the increased production of cyclic AMP by these agents in rat adipose tissue[4]. The lipolytic effect of DHE itself in isolated rat fat cells is very slight but in dog fat cells, the intrinsic activity of DHE is almost as great as that of NE and its affinity for the lipolytic system is even higher than that of NE. It was also important to see that more than 100 times the concentration of propranolol required to inhibit NE-induced lipolysis was necessary to block the DHE-induced lipolysis. Our experiments demonstrate that DHE increased significantly the adenyl-cyclase activity in the dog fat cells 'ghosts', the cyclic AMP production induced by DHE being lower than with NE stimulation.

By examining the results obtained with DHE and propranolol in the dog, we found some resemblance between this effect and the action of β-blockers on polypeptide-induced lipolysis, in particular ACTH, in the rat fat cells[18,19], but it was not possible to compare directly ACTH and DHE in the dog, because ACTH has no lipolytic action in dog fat cells[20].

On the basis of these observations, it is conceivable that DHE might not act on receptors of adenyl-cyclase for catecholamines. It activates perhaps the adenyl-cyclase system by changing the conformation of the cell membrane or reacting with the receptors of adenyl-cyclase for polypeptides.

Zusammenfassung. Die Beeinflussung der Lipolyse isolierter Fettzellen des Hundes durch Dihydroergotamin (DHE) wurde untersucht. DHE stimuliert die Lipolyse ähnlich wie Noradrenalin. Insulin und Propranolol antagonisieren DHE. Die Adenyl-cyclase wird stimuliert, die Phosphodiesterase wird hingegen durch DHE nicht beeinflusst.

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