and feeding them a low fat diet the problems of cannula blockade by clotting and chylomicra occlusion were minimized. Using this technique, over 50% of the animals operated upon can be expected to drain for prolonged periods of time.

Summary. A simple method for cannulating the mouse thoracic duct is described that enables the collection of lymph for periods of up to five days. A pure lymphocyte population is obtained that is useful for immunologic and antibody synthesis studies.

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Development of Tachyphylaxis to the Antilipolytic, Hypoglycemic Agent, 5-Methylpyrazole-3-carboxylic Acid, U-19425.* (32495)

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Acute hypoglycemic and antilipolytic effects of 5-methylpyrazole-3-carboxylic acid (U-19425) in rats have been described(1). U-19425 decreased blood sugar acutely in alloxan-diabetic rats, but tachyphylaxis was suspected since U-19425 did not have a sustained effect on glucosuria of these animals.

This report describes development of tachyphylaxis to U-19425. U-19425 pretreatment results in a more rapid return of plasma FFA to control levels after a challenge dose of the pyrazole. The data suggest that lipolytic enzyme systems of pretreated rats escape faster from the inhibitory effect of U-19425. This faster return of plasma FFA to normal is related to adrenalcortical secretion.

Methods. Studies on development of tachyphylaxis to U-19425 were done in intact male Spartan Sprague-Dawley rats weighing 150-160 g. Since hormones influence lipid metabolism(2), studies were also done in adrenalectomized and hypophysectomized rats. Rats were adrenalectomized at a weight of 150-170 g two days prior to the start of pretreatment. Adrenalectomized animals were maintained on 0.9% saline drink from time of operation until termination of the experiment. Studies on the effect of hydrocortisone replacement on tachyphylaxis were also done in adrenalectomized rats. Half of the adrenalectomized animals received a single daily subcutaneous injection of 0.5 mg of hydrocortisone while the other half received saline during the 4-day pretreatment period.

Rats were hypophysectomized by the transauricular approach of Koyama(3) as modified by Falconi and Rossi(4). Rats were injected with 150 μg of prednisolone per day for the first 2 days and maintained on 5% sucrose drink for the first 10 days after hypophysectomy. Rats which did not gain weight were used for tachyphylaxis studies 2 weeks after hypophysectomy.

* Cyclopal is The Upjohn Co., registered trademark for 5-allyl-5-(2-cyclopenten-1-yl) barbituric acid.

Rats were pretreated orally b.i.d. with 0.5 ml of either carboxymethylcellulose vehicle or U-19425 for 4 days. After the last dose on day 4, animals were fasted overnight. On the morning of the fifth day, half of each pretreated group was challenged with U-19425 and half with vehicle. Immediately following oral treatment on day 5, all rats were primed with a subcutaneous injection of 125 mg of glucose in 1 ml saline.

Blood was withdrawn from the posterior vena cava while the animals were under Cyclopal®* sodium anesthesia. Blood glucose was determined by the AutoAnalyzer which utilizes the ferricyanide reduction procedure of Hoffman(5). Plasma free fatty acids (FFA) were measured by the Dole technique(6) as modified by Trout *et al*(7).

All data were evaluated statistically by either Dunnett's test(8) or Student's t test (9).

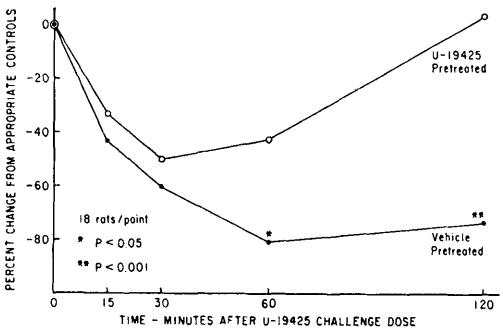
Results. Four days of pretreatment with U-19425 completely blocked the blood sugar and plasma FFA response 2 hours after the challenge dose of U-19425 (Table I). However, 4 days of U-19425 treatment did not alter basal fasting blood sugar or plasma FFA levels 18 hours after last treatment.

During the first 30 minutes after the challenge dose of U-19425, the percent change in plasma FFA levels was similar in both U-19425 and vehicle-pretreated rats (Fig. 1). The percent change in U-19425-challenged animals was calculated from their appropriate controls. Thirty minutes after the challenge dose, FFA of U-19425-pretreated animals started to return toward normal and reached control levels in 2 hours; but FFA of vehicle-pretreated rats continued to fall to —80% of control at one hour and were still depressed 2 hours after the challenge dose. Blood sugar levels of the same U-19425-pretreated rats did not fall significantly at any time during the 2 hours after the challenge dose of U-19425, but blood sugar levels of the vehicle-pretreated rats did

TABLE I. Effect of U-19425 on Blood Sugar and Plasma FFA of Pretreated, Fasted, Glucose-Primed Rats. Measurements made 2 hours after challenge dose on day 5.

		Days 1-4		Day 5			
Group No.	No. Rats	Treatment	Dose (mg/kg b.i.d.)	Challenge	Dose (mg/kg)	Blood sugar (mg% ± S.E.*)	Plasma FFA (μE/l ± S.E.*)
A	11	Vehicle	—	Vehicle	—	71 ± 2.1	676 ± 27.5
B	11	"	—	U-19425	1.0	49 ± 2.7†	396 ± 42.9†
C	11	U-19425	5.0	Vehicle	—	72 ± 2.2	731 ± 40.3
D	11	"	5.0	U-19425	1.0	66 ± 2.8	705 ± 78.6

* Standard error of the mean.
† $P < .001$, B *vs* A, B *vs* D, sugar and FFA.
C *vs* D, not significant, either sugar or FFA.
A *vs* C, not significant, either sugar or FFA.



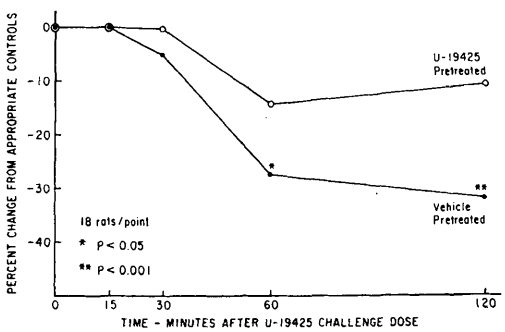


FIG. 1. Effect of U-19425 challenge dose on plasma FFA of U-19425 and vehicle-pretreated rats.

FIG. 2. Effect of U-19425 challenge dose on blood sugar of U-19425 and vehicle-pretreated rats.

TABLE II. Effect of U-19425 Pretreatment and Hydrocortisone Therapy on the Response of Adrenalectomized Rats to U-19425. Measurements made 2 hr after challenge dose on day 5.

Group No.	No. Rats	Days 1-4 Treatment	Dose (mg/kg b.i.d.)	Day 5 Challenge	Dose (mg/kg)	Blood sugar (mg% ± S.E.*)	Plasma FFA (μE/l ± S.E.*)
				Saline, subcutaneous			
A	11	Vehicle	—	Vehicle	—	71 ± 1.5	616 ± 48
B	12	"	—	U-19425	1.0	14 ± 1.6†	258 ± 27†
C	12	U-19425	5.0	Vehicle	—	69 ± 3.9	466 ± 53
D	12	"	5.0	U-19425	1.0	22 ± 0.3†	251 ± 34†
				Hydrocortisone (0.5 mg), subcutaneous			
E	11	Vehicle	—	Vehicle	—	72 ± 1.8	536 ± 21
F	12	"	—	U-19425	1.0	14 ± 2.8†	300 ± 25†
G	12	U-19425	5.0	Vehicle	—	76 ± 2.7	617 ± 58
H	12	"	5.0	U-19425	1.0	60 ± 7.0	621 ± 94

* Standard error of the mean.
† $P < .01$, B *vs* A, D *vs* C, F *vs* E, sugar and FFA. All other statistical comparisons were made but no significance observed.

fall significantly after the challenge dose of U-19425 (Fig. 2).

Adrenalectomy prevented the development of tachyphylaxis to U-19425 (Table II). Administration of hydrocortisone to adrenalectomized rats pretreated with U-19425 resulted in tachyphylaxis similar to that observed in pretreated, intact rats. Hypophysectomy also prevented the development of tachyphylaxis to U-19425 (Table III).

Discussion. Acutely, U-19425 is a very potent hypoglycemic and antilipolytic agent in rats(1). However, tachyphylaxis to the pyrazole develops within 4 days.

Although the mechanisms responsible for tachyphylaxis are unknown, it is not due to altered metabolism of the pyrazole since Smith *et al*(10) have shown that U-19425-C^{14} metabolism and excretion is the same in U-19425-pretreated and nonpretreated rats. The decreased duration of plasma FFA depression in pretreated animals is not due to increased basal release of FFA since adipose tissues from pretreated and nonpretreated rats had similar lipolytic rates when studied *in vitro* (unpublished results) and fasting plasma FFA levels are the same in pretreated and nonpretreated rats.

As previously postulated(1), blood sugar depression due to U-19425 may be secondary to an initial decreased availability of plasma FFA. If this hypothesis is correct, a lack of hypoglycemia in the rat treated with pyrazole for 4 days might be expected since the duration of FFA lowering is reduced.

Tachyphylaxis to 5-methylpyrazole-3-carboxylic acid is due to a more rapid escape from its inhibitory effects on lipolysis. The more rapid rebound of plasma FFA to a normal level may be due to a physiological compensatory mechanism essential for survival since peripheral tissues of the fasting animal are dependent on plasma FFA for energy. The data show that tachyphylaxis to U-19425

TABLE III. Effect of U-19425 Pretreatment on Response of Hypophysectomized Rats to a Challenge Dose of U-19425. Measurements made 2 hours after challenge dose on day 5.

Group No.	No. Rats	Days 1-4 Treatment	Dose (mg/kg b.i.d.)	Day 5 Challenge	Dose (mg/kg)	Blood sugar (mg% ± S.E.*)	Plasma FFA (μE/l ± S.E.*)
A	5	Vehicle	—	Vehicle	—	47 ± 3.4	452 ±106
B	5	"	—	U-19425	1.0	32 ± 3.3†	104 ± 31†
C	6	U-19425	5.0	Vehicle	—	59 ± 4.4	431 ± 34
D	5	"	5.0	U-19425	5.0	37 ± 2.3†	144 ± 16†

* Standard error of the mean.
† $P < .01$, B *vs* A, D *vs* C, sugar and FFA. A *vs* C, B *vs* D not significant.

is related to adrenal and/or hypophyseal function. Removal of these glands abolishes tachyphylaxis, but the adrenalectomized, hydrocortisone-treated rat does develop tachyphylaxis. Therefore, this physiological compensatory mechanism is mediated by adrenal and/or hypophyseal hormones. This suggestion is consistent with the findings of Goodman and Knobil(2) who found that the pituitary and adrenal cortex are not necessary for lipid mobilization during fasting, but the lipolytic response is reduced following ablation of these glands and cortisol administration to adrenalectomized rats returned the lipolytic response to normal. The postulated role of the adrenal and pituitary glands in development of tachyphylaxis to U-19425 is further supported by Pereira's findings in nicotinic acid-treated, adrenalectomized, hypophysectomized rats(11). He found that the characteristic plasma FFA rebound to levels higher than normal after nicotinic acid did not occur if both glands were removed, but FFA rebound above normal if only one or the other was removed.

Summary. After 4 days of treatment with U-19425, rats no longer respond to the drug with a decrease in plasma FFA and blood sugar 2 hours after treatment. The initial plasma FFA response to the drug is similar, but pretreated rats escape from antilipolytic effects of U-19425 faster than nonpretreated controls. Tachyphylaxis to 5-methylpyrazole-3-carboxylic acid may be due to a physiological compensatory mechanism which is mediated by the adrenal and/or pituitary glands.

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Intracellular Localization of 3-4 Benzo(a)Pyrene in *Saccharomyces cerevisiae.* (32496)

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The yeast, *Saccharomyces cerevisiae,* was used as a test cell to investigate the intracellular localization of Benzo(a)pyrene. The localization was investigated primarily by ultraviolet microscopy, and the observations were correlated with intravital staining with Janus Green B and other techniques.

Materials and methods. Saccharomyces cerevisiae (strain 7921 American Type Culture Collection) was cultivated and tested in Middlebrook 7H9 broth base with 5% (w/v) Dextrose.

Organisms were grown aerobically in the dark at 35°C in glass 20 × 150 mm screw capped tubes.

Benzo(a)pyrene (Aldrich Chemical Co., Milwaukee, Wis.) was purified in a darkened room by paper chromatography on Whatman No. 1 filter paper using solvents prepared by the method of Lijinsky and Raha (1). Spectral characteristics of the purified eluants were identical with standards of pure Benzo(a)pyrene prepared in this laboratory. A stock solution containing 2 mg