tems. It is equally important that dependable methods of elimination be available. Little is known of the mechanisms by which these contaminants may alter the results of physiological and virological studies using mammalian cells *in vitro,* but there is mounting evidence that effects do occur(5,6,18,19), and it behooves the investigator to use every means at his command to control the problem.

Summary. A procedure for control of PPLO contamination in mammalian cell cultures has been presented as an alternative to the employment of antibiotics. Six cell lines have been freed of PPLO by comparatively short-term exposure to antiserum specific for the contaminant in question. For destruction of 2 of the mycoplasma species a heat-labile serum component was required; the remaining 4 lines were successfully treated with inactivated serum. Because control of these contaminants is of utmost importance in the use of cell cultures as experimental systems application of this technic may be advantageous when antibiotic resistance is encountered.

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Tachyphylaxis to Epinephrine and Its Modification by Cocaine.* (27986)

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Seevers and Woods(1), reviewing the phenomena of tolerance to pharmacological agents concluded that "no tachyphylaxis is developed to the pressor effects of epinephrine in the dog." This conclusion was based largely on the experimental findings of Winder *et al.*(2) and Essex(3). We have found no later evidence in the literature to alter the conclusions of Seevers and Woods, that tachyphylaxis to the pressor effects of epinephrine cannot be established. On the other hand Rosenthal and Di Palma(4) have demonstrated the development of tachyphylaxis to norepinephrine in the dog. Their findings stimulated us to investigate the possibility of producing acute tolerance to epinephrine.

In the experiments to be reported we have studied: (a) the experimental conditions under which tachyphylaxis to epinephrine can

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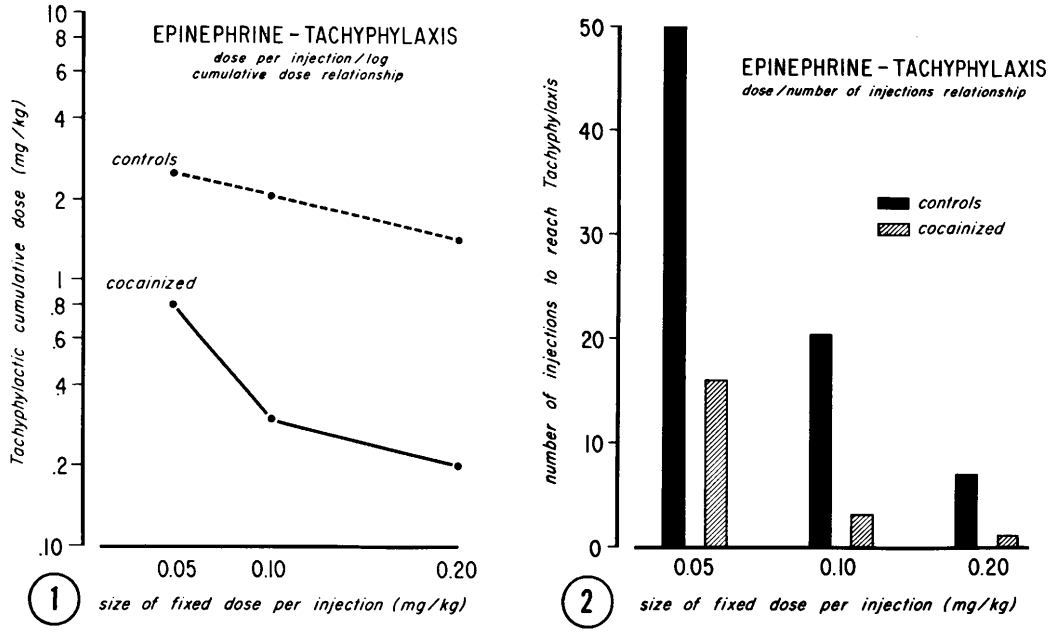

FIG. 1. Relationship between logarithm of cumulative dose and size of fixed dose per injection in production of tachyphylaxis.

FIG. 2. Relationship between size of fixed dose per injection and number of injections required for tachyphylaxis.

be obtained and modified; (b) its reversibility; and (c) the protective effect of tachyphylaxis against injection of toxic doses of epinephrine.

Methods. In animals under ether or intraperitoneal urethane-chloralose anesthesia (urethane 700 mg/kg; chloralose 35 mg/kg), the left femoral vein and artery were catheterized. A system of Statham transducers and a type RP Offner polygraph was used for the continuous recording of arterial blood pressure and respiration.

Repeated administration of epinephrine in large doses frequently results in profound cardiac and respiratory irregularities. To obviate the cardiac effects the animals were either vagotomized or atropinized. Tracheotomies were performed so that the animals could be maintained under artificial respiration when necessary.

Induction of tachyphylaxis was performed by rapidly injecting freshly prepared solutions of epinephrine into the catheterized left femoral vein. The volume of injection was kept constant (1 ml). This was injected within 10 seconds at intervals of 5 minutes in all experiments. After each injection the system was flushed with 2.5 ml of normal saline solution.

Three different doses of epinephrine per injection were used: 0.05 mg/kg (10 cats); 0.10 mg/kg (4 cats); and 0.20 mg/kg (2 cats). Half of the cats received 5 mg/kg of cocaine HCl dissolved in 5 ml of normal saline, intraperitoneally 30 minutes prior to the beginning of epinephrine injections (Fig. 1).

When the group of cats repeatedly injected with 0.05 mg/kg of epinephrine were rendered tachyphylactic, massive single doses of this drug (1 and 5 mg/kg) were given intravenously to test whether the tachyphylactic state affords protection to acute epinephrine toxicity. The animals were observed for one hour after which they were sacrificed.

In the group of 6 cats receiving larger doses of epinephrine per injection (0.10 and 0.20 mg/kg), respiratory irregularities with marked periods of apnea invariably followed. Consequently all animals in this group were maintained on artificial respiration. After tachyphylaxis ensued the epinephrine injections were discontinued. One hour later the reversibility of the tachyphylaxis was determined by measuring the pressor response to a small

TABLE I. Comparison of Doses of Epinephrine Required for Tachyphylaxis in Control and Cocainized Cats.

Size of fixed dose per injection mg/kg	Control animals tachyphylaxis			Cocainized animals tachyphylaxis		
	No. of cats	Avg number of injections	Avg cumulative dose mg/kg	No. of cats	Avg number of injections	Avg cumulative dose mg/kg
.05	5	50	2.5	5	16	.80
.10	2	20.5	2.05	2	3	.30
.20	1	7	1.4	1	1	.30

amount of epinephrine (0.01 mg/kg) and comparing it to the response to an identical dose injected prior to induction of tachyphylaxis.

Results. Tachyphylaxis to epinephrine. In our first experiments we observed that as the number of injections increased the pressor response to epinephrine gradually decreased to a point at which no further response was elicited, even with larger doses than the ones previously utilized. However, to reach this point of absolute tachyphylaxis required so many injections and so prolonged an experiment that it was decided to consider that tachyphylaxis had been achieved, in a relative sense, when the magnitude of the mean systolic blood pressure response to 5 consecutive injections of epinephrine was less than 33% of the mean systolic blood pressure response of the first 5 injections.

In the group of 5 control cats receiving 0.05 mg/kg of epinephrine per injection, a mean number of 50 injections were required for induction of tachyphylaxis. The average cumulative dose of epinephrine was 2.5 mg/kg. Under these conditions no cases of respiratory failure occurred and artificial respiration was not necessary (Table I).

The pre-epinephrine injection blood pressure levels decreased from an initial mean value of 148/85 to 123/64 mm Hg when tachyphylaxis was reached. This finding indicates that the decreased pressor response observed after repetitive administration of epinephrine is not the consequence of a gradual elevation of pre-injection blood pressure levels, but rather a decreased pressor response to the injected catecholamine. The results clearly demonstrate that tachyphylaxis to epinephrine can be made to occur.

All of the animals utilized in the previous experiments were intravenously injected with 1 and 5 mg/kg of epinephrine, at the height of tachyphylaxis. A transient period of hypotension and hypopnea invariably followed, from which the animals recuperated spontaneously. No animal died with these massive doses. Tachyphylactic animals are therefore able to tolerate single doses of epinephrine which are rapidly lethal to animals in whom tachyphylaxis has not yet been induced. *Effect of cocaine.* Following cocainization tachyphylaxis was obtained after a mean number of 16 injections in the group of 5 cats receiving 0.05 mg/kg of epinephrine per injection. The average cumulative dose of epinephrine was 0.80 mg/kg. The differences between control and cocainized groups were investigated statistically by a "t test" and were found to be significant at 0.001 level of confidence (Table I, Fig. 1).

The results clearly demonstrate that cocaine accelerates the development of tachyphylaxis to epinephrine. As in the control group the cocainized tachyphylactic animals were protected against massive single injections of epinephrine (1 and 5 mg/kg) and tachyphylaxis occurred as a consequence of a decreased pressor response to the epinephrine injected and not to changes in the basal blood pressure level.

Influence of size of fixed dose. Tachyphylaxis was induced in 3 groups of cats by injecting repetitively 0.05, 0.10 and 0.20 mg/kg of epinephrine respectively. The number of injections and the total cumulative dose required for tachyphylaxis was determined (Table I). The results show that increases in size of dose per injection were inversely related to the logarithm of the cumulative dose and to the number of injections required to reach tachyphylaxis (Figs. 1, 2). This relationship was common to both control and cocainized animals, the latter requiring considerably fewer injections to reach tachyphylaxis at every dose level.

Reversibility of tachyphylaxis. The mean systolic blood pressure response of 6 cats tested with a small amount of epinephrine (0.01 mg/kg) before induction of tachyphylaxis was 69 mm Hg. At the peak of the tachyphylactic state no response was obtained. One hour later the response was 38 mm Hg, or 55% of the initial pressor response. These findings are interpreted to indicate that tachyphylaxis to epinephrine is a rapidly reversible phenomenon.

Discussion. It is understandable that tachyphylaxis to epinephrine has not been previously reported because so large a quantity and so many injections are required to reach it. Furthermore, if other workers employed doses smaller than 0.05 mg/kg, tachphylaxis to epinephrine would take an even longer time to develop or might not develop at all. In one of our pilot experiments preceding this study, we tested a dog with a fixed dose of 0.01 mg/kg of epinephrine. After 155 injections (at 5 minute intervals each), this dog showed no evidence of becoming tachyphylactic. On the other hand 2 dogs tested in our pilot studies with epinephrine injections of 0.05 mg/kg at 5 minute intervals reached tachyphylaxis after a mean number of 17.5 injections. This is more rapid than the results obtained with the cats.

Our results give support to the "receptor saturation" theory of tachyphylaxis. Thus, this theory demands: (a) that the saturation of receptors and therefore the speed at which tachyphylaxis occurs should be a function of drug concentration; and (b) that saturation of a receptor should exert no effect other than to prevent the initiation of a new response by receptor combination with other molecules. We have found that the number of injections required to reach tachyphylaxis is inversely related to the size of the repetitive dose of epinephrine used, and that basal blood pressure levels throughout the development of tachyphylaxis are stable or decrease only slightly, while the pressor response to epinephrine steadily decreases.

Although our reported data deal only with systolic blood pressure responses, tachyphylaxis to the diastolic blood pressure response to epinephrine also occurs. Control cats required a mean number of 59 injections and the cocainized group a mean number of 22 injections, to reach diastolic tachyphylaxis ($p = 0.005$).

Our results demonstrating the acceleration of tachyphylaxis to epinephrine by cocaine are unequivocal. The mechanism is less clear because the mode of action of cocaine is still controversial. The early views that cocaine directly stimulates or sensitizes sympathetically innervated structures has been repeatedly refuted(5). Similarly the view that cocaine stimulates the production of sympathomimetic amines at nerve endings is unsupportable. The two hypotheses that have received more attention are: (a) that cocaine delays the inactivation of circulating sympathomimetic amines(6,7), and (b) that cocaine interferes with a mechanism that limits the concentration of active circulating catecholamines. This limiting mechanism has been attributed either to the capacity of tissues innervated by sympathetic nerve endings to store catecholamines(8,9) or to the removal of these amines by the adrenergic nerve terminals themselves(10).

The first hypothesis has received both *in vitro*(11) and *in vivo* support(12), but is difficult to reconcile with the work of Axelrod and Tomchick(13) who failed to find any significant effect of cocaine upon the metabolic rate of tritium labelled epinephrine in mice. The evidence for or against this hypothesis is not conclusive, and certainly such an explanation cannot be discarded as a possibility. Nevertheless, we favor the second hypothesis not only because of the accumulated positive evidence but also because of the absence of contradictory data. Thus, Stromblad and Nickerson(14), studying accumulation of epinephrine and norepinephrine by some rat tissues, concluded that these two catecholamines can be stored in tissues innervated by sympathetic nerve endings, and that such tissues have a storage capacity considerably greater than their normal catecholamine content.

Although in our experiments we have not determined the levels of circulating catecholamines, it seems well established that the amount of exogenous circulating epinephrine is greater following cocainization. Whitby *et al.*(15) have demonstrated that in cocainized

animals administration of tritium labelled epinephrine and norepinephrine results in a higher plasma concentration and a lower tissue concentration of these amines than in control animals. These experiments have been confirmed by Muscholl(16) and Hertting *et al.* (17).

Whether cocaine blocks the "storage" of catecholamines by tissues innervated by sympathetic nerve endings or "removal" of these amines by adrenergic nerve terminals themselves is not clear. The action of cocaine, however, indicates the existence of some similar mechanism serving as an homeostatic system, which by reducing the concentration of active circulating catecholamines moderates their physiological effects.

Although the existence of such an homeostatic system was suspected as an explanation of the potentiating action of cocaine upon exogenous epinephrine, its quantitative significance could not be judged as clearly as it is revealed in the accelerated tachyphylaxis to epinephrine. Thus, inspection of our results (Fig. 1) shows that control animals required, respectively, 1.7, 1.75, and 1.1 mg/kg of epinephrine more than the cocainized cats, at each of the repetitive doses used (0.05, 0.10, and 0.20 mg/kg), before saturation of the receptors occurred. Therefore, animals in which the catecholamines regulating mechanism is intact can "moderate" large amounts of epinephrine before the adrenergic receptors saturate and become tachyphylactic. In animals in which this mechanism is blocked by cocaine, the adrenergic receptors are exposed to a many-fold increase in the number of epinephrine molecules allowing initially a more vigorous response (potentiation) and earlier saturation (accelerated tachyphylaxis).

Summary and conclusions. 1) It has been demonstrated that tachyphylaxis to epinephrine, in contradiction to earlier reports, can be made to occur both in cats and dogs; that repeated injections of large doses are required; that at a constant rate of administration, the logarithm of the tachyphylactic cumulative dose is inversely related to the size of the fixed dose per injection; that tachyphylaxis occurs only at the expense of the pressor response to the drug and not to changes in basal blood pressure level; that the tachyphylactic animal, whether cocainized or not, is protected against otherwise single lethal doses of epinephrine; and that tachyphylaxis is a reversible phenomenon. 2) It was found that cocaine very significantly accelerates the development of tachyphylaxis to epinephrine. This finding is interpreted as furnishing evidence for the existence of an important homeostatic mechanism which, by reducing the concentration of active circulating catecholamines moderates their physiological effect. This limiting mechanism has been attributed either to the capacity of tissues innervated by sympathetic nerve endings to store catecholamines or to removal of these amines by the adrenergic nerve terminals themselves. It is suggested that cocaine acts by blocking this homeostatic mechanism. As a consequence a greater concentration of circulating catecholamines occurs in the cocainized animal, its adrenergic receptors are exposed to a larger number of molecules of these amines, allowing the receptors to trigger a more vigorous response (potentiation) and to become saturated earlier (accelerated tachyphylaxis).

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Relationship Between Deuterium Inhibition of Growth and Glucose Catabolism in *Saccharomyces cerevisiae*.* (27987)

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The biological effects of deuterium have been ably reviewed by Morowitz and Brown (1) and more recently by Katz(2). From these reports it is evident that almost without exception cell division is partially or completely inhibited by deuterium. Sensitivity to deuterium varies considerably among different types of cells and apparently is related to the mechanism by which cell division is accomplished. Mitosis is completely inhibited at all stages in 50 to 75% deuterium oxide (D_2O)(3,4), whereas bacteria, yeast, and some algae, which accomplish cell division by other means, can be grown in 99+% D_2O(5). Deuterium inhibition of microbial cell division (growth) is thought to be due to effects on the metabolic state of the cell(3), although direct experimental evidence for this is lacking. In view of this, the effects of deuterium on growth and glucose catabolism in yeast were compared.

Methods. A haploid strain of *Saccharomyces cerevisiae* was used for these studies. Stock cultures were maintained in peptone, yeast extract, glucose agar slants and were transferred at monthly intervals. For experiments cells were cultured at 25°C in the defined medium of Burkholder, McVeigh and Moyer(6). This medium supported growth when made with D_2O or water; however, for comparative purposes in some experiments the medium was supplemented with 1% peptone and 0.5% yeast extract. Before use D_2O was distilled with permanganate and the final concentration as determined by specific gravity was 99-99.5%. Growth was followed routinely by determining the optical densities of cultures at 640 mμ on a Coleman spectrophotometer and by periodic dry weight determinations. Standard manometric technics (7) were employed in gas exchange studies. All experiments were repeated at least 5 times and results are reported as mean ± standard deviation.

Results. Growth studies. In Fig. 1 typical results of growth experiments are presented. Curve A shows the growth pattern seen with water grown cells in water media and serves as a control. Curve C illustrates the inhibitory effects of D_2O on growth of cells previously grown in water. The initial lag period was prolonged in D_2O media, in this case being about 30 minutes compared to 15 minutes in water media. The lag phase in D_2O was always about twice as long as in water regardless of composition of the medium. During log growth optical density increased 0.07 ± .01 O.D. units per hour in water media compared to .03 ± .01 units per hour in D_2O media. When media were supplemented with peptone and yeast extract growth rates increased; however, the relative deuterium effect $\left(\frac{\text{growth rate in } D_2O}{\text{growth rate in } H_2O} = 0.47 \pm .07\right)$ was the same. Attempts to follow growth by direct cell counting were unsuccessful due to clumping of the cells, particularly in D_2O cultures.

Also shown in Fig. 1 are results of experiments in which cells previously grown in D_2O were subcultured in D_2O and ordinary water media. Curve D shows the growth pattern of deuterated cells in D_2O, and by comparing this curve with Curve C (water grown cells in D_2O), it may be seen that growth rates were the same in each case (0.03 O.D.

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