show no increase in number from day 7 through day 21. Increase in size from 1 mm at day 7 to 3-4 mm occurs over the 21-day period. The last 4 days several bottles were incubated upright, and the plaques developed long necrotic streamers in the direction of gravity. The control cell cultures appear relatively normal during the entire period of incubation and are apparently viable as evidenced by the increase in plaque size. The only change noted in microscopic morphology is the increased granularity of the normal cells. The plaques microscopically (28× magnification) have a hyaline to necrotic appearance, and many have hollow centers.

*Challenge of other host cells with tissue culture virus.* Sonicated, undiluted preparations from the 10th and 25th passages were inoculated intraperitoneally into hamsters 3 weeks of age, and resulted in death in 3-6 days. Sections of the livers of these animals showed characteristic lesions(5). Materials diluted $10^{-2}$ sickened but did not kill animals. This is somewhat similar to the reactions obtained with HeLa passage virus(1). Dilution of L-passage virus diluted $10^{-5}$ regularly produced characteristic cytopathic effect in HeLa cell monolayers with typical intranuclear inclusions.

*Discussion and summary.* This report is an account of the adaptation of EAV to L-M 929 cells with identification by complement-fixation methods. Infected cultures show cytopathic effects which may be measured by both $TCID_{50}$ or plaque-counting methods. The failure of the virus to propagate in L cells (original) in early experiments is unexplained. During this period the cells were grown in horse and human sera which may have been inhibitory. The possibility that the L-M 929 strains could be directly infected by hamster-propagated EAV has not been excluded. The possibility of contamination of the L-cell strain used in this study with HeLa cells has been considered. The special chromosomal marker of the L-M 929 strain permits precise identification and has not changed, according to Hsu and Merchant(6). Furthermore, YELP has proven very toxic to the HeLa cell line, and cell growth is highly unlikely in this medium.

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## Early Potentiation of the Vasoconstrictor Action of Norepinephrine by Guanethidine.* (27559)

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The work of several authors(1-4) indicates that the pressor response to norepinephrine increases after intravenous administration of guanethidine. It is not clear from these observations whether the increased pressor response is caused by a greater cardiac output or by augmentation of the vasoconstrictor effect of the norepinephrine. The experiments to be reported here were done to determine the effect of norepinephrine on blood flow and vascular tone before and after intravenous

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guanethidine in healthy men and intact dogs.

*Material and methods.* In the first group of experiments 9 dogs were lightly anesthetized with thiopental sodium, treated with gallamine triethiodide and ventilated with a fixed-volume respiratory pump through a cuffed endotracheal tube. Cardiac output was measured by the indicator-dilution technic with injections of indocyanine green dye into the main pulmonary artery through an intracardiac catheter. Dyed blood was drawn from the carotid artery through the cuvette of a Gilford densitometer. Blood pressure was measured with a Statham strain-gauge connected to a needle in the femoral artery. Dye curves and blood pressure were recorded with a Sanborn direct-writing oscillograph. Norepinephrine bitartrate was infused intravenously at rates of 0.15, 0.30 and 0.60 μg of base/kg/minute in ascending order. Each infusion lasted 6 minutes and was followed by a 20-minute recovery period. Observations on cardiac output and blood pressure were made immediately before and at the end of 3 minutes of infusion. Guanethidine (1 mg/kg) was injected intravenously after the control observations were made. The 3 infusions of norepinephrine were repeated beginning 30 minutes after the guanethidine injection. Cardiac output was calculated from the dye curves using the Stewart-Hamilton equations (5,6). Peripheral resistance was calculated by dividing the mean arterial pressure in mm Hg by cardiac output in l/minute; it is expressed in arbitrary units.

Five healthy young men served as subjects for the second group of experiments. They were studied while lying in the supine position. Blood flow was measured in the left forearm using a water plethysmograph(7). Brachial arterial pressure was measured in the right arm by auscultation. Observations on blood flow and arterial pressure were made before and during each of 3 infusions of norepinephrine bitartrate given at rates of 0.075, 0.15 and 0.30 μg of base/kg/minute in ascending order. Each infusion lasted 6 minutes and was followed by a 20-minute recovery period. After the control observations were made guanethidine, 0.25 mg/kg in 100 ml of 5% glucose in water, was injected intravenously in 10 minutes. The 3 infusions of norepinephrine were repeated in the same fashion beginning 15 minutes after guanethidine injection. Blood flow was calculated from rate of increase in arm volume during venous occlusion and expressed in ml/100 ml of forearm volume/min. A wrist cuff was inflated to levels above arterial pressure during measurement of forearm flow. At least 3 flow curves were recorded during each minute. The resting values (Table II) represent averages of all values obtained during the 3-minute periods before each infusion of norepinephrine. The experimental values are averages of all observations made between the beginning of the third and the end of the sixth minute of infusion. The reported blood pressures were obtained at the same time as the blood flows. The mean blood pressure (diastolic plus one-third of the pulse pressure) was divided by the corresponding blood flow to obtain an expression of forearm vascular resistance in arbitrary units.

The experiments were designed to compare the responses to norepinephrine before and after guanethidine. Dose-response regressions were computed for mean arterial pressure, cardiac output and resistance in the dogs and for mean arterial pressure, forearm blood flow and resistance in the men. Whenever the regressions before and after guanethidine were linear and parallel and satisfied other requirements for a parallel line bioassay(8), the "relative potency"§ of norepinephrine was calculated. When the effect of norepinephrine was potentiated significantly after guanethidine, the "relative potency" and its lower 95% fiducial limit were greater than 1.0. When the requirements for the bioassay were not satisfied, the responses to norepinephrine were compared using Student's t-test(9).

*Results.* In dogs, the values for systemic arterial pressure and peripheral resistance obtained with each dose of norepinephrine were higher after intravenous guanethidine (Table I). The effect of norepinephrine on cardiac

§ The ratio of a dose of norepinephrine which gave a certain response before guanethidine to the dose which caused the same response after guanethidine.

TABLE I. Effects of Graded Doses of Norepinephrine before and after Guanethidine in 9 Dogs.

| | Mean blood pressure, mm Hg | | | Cardiac output, l/min. | | | Peripheral resistance, units | | |
|---|---|---|---|---|---|---|---|---|---|
| | B | A | | B | A | | B | A | |
| Resting values | 130.1 ± 3.9 | 120.6 ± 5.6* | | 2.3 ±.2 | 1.9 ±.2* | | 59.8 ± 5.0 | 71.9 ± 8.5* | |
| Dose of norepinephrine (μg/kg/min.) | | | | | | | | | |
| .15 | 132.6 ± 2.6 | 154.2 ± 7.8 | ‡ | 2.3 ±.1 | 1.9 ±.2 | † | 59.0 ± 5.3 | 88.4 ± 8.5 | * |
| .30 | 145.6 ± 5.6 | 171.0 ±10.0 | | 2.3 ±.2 | 2.1 ±.2 | | 65.3 ± 6.1 | 89.1 ±10.8 | |
| .60 | 176.7 ± 9.7 | 199.1 ±10.4 | | 2.4 ±.2 | 2.3 ±.2 | | 82.2 ±10.3 | 97.4 ±11.5 | |

The entries represent mean values and 1 stand. error of mean for observations in all 9 dogs at rest and during each norepinephrine infusion. B = values obtained before guanethidine; A = values obtained after guanethidine.

* and † indicate significance of the difference between A and B using the t-test. * $P < .05$. † $P > .05$.

‡ "Relative potency" (RP) of norepinephrine was 2.1. Its 95% fiducial limits were 3.9 and 1.4.

output was negligible and was not altered significantly by guanethidine. In men, the potency of norepinephrine with respect to its effect on mean arterial pressure was significantly greater after guanethidine (Table II). Forearm blood flow was somewhat higher during the norepinephrine infusions after guanethidine, but vascular resistance increased to levels similar to those observed before the guanethidine. The resting mean values for blood pressure, cardiac output and resistance in dogs and blood pressure, forearm blood flow and resistance in men are in Tables I and II.

*Discussion.* In dogs, resting blood pressure and cardiac output were reduced and peripheral resistance was increased 30 minutes after guanethidine. This increase in resistance does not necessarily indicate an increase in vascular tone. It may have been caused by the fall in distending pressure with passive reduction in vascular caliber(10). When the infusions of norepinephrine were repeated during this hypotensive period, both blood pressure and resistance reached higher levels than before guanethidine. This must mean that the effect of norepinephrine on vascular tone was augmented.

It is interesting that guanethidine increased forearm blood flow and decreased forearm vascular resistance in 4 of the 5 subjects. It had little effect on blood pressure. Increasing doses of norepinephrine caused progressive increases in blood pressure and forearm vascular resistance with progressive decreases in blood flow. After guanethidine, the effect of norepinephrine on blood pressure was augmented while its effect on vascular resistance was unchanged. Vascular tone must have increased when resistance remained un-

TABLE II. Effects of Graded Doses of Norepinephrine before and after Guanethidine in 5 Men.

| | Mean blood pressure, mm Hg | | | Forearm blood flow, ml/min./100 ml | | | Vascular resistance, units | | |
|---|---|---|---|---|---|---|---|---|---|
| | B | A | | B | A | | B | A | |
| Resting values | 84.3 ± 2.8 | 86.4 ± 1.9† | | 5.1 ±.5 | 6.3 ±.5† | | 17.0 ± 1.8 | 14.1 ± 1.1† | |
| Dose of norepinephrine (μg/kg/min.) | | | | | | | | | |
| .075 | 92.6 ± 3.6 | 98.9 ± 1.0 | ‡ | 5.1 ±.5 | 5.7 ±.6 | § | 19.8 ± 3.4 | 18.2 ± 2.6 | ‖ |
| .15 | 100.7 ± 3.7 | 111.6 ± 2.0 | | 4.1 ±.2 | 4.6 ±.4 | | 24.7 ± 1.7 | 24.7 ± 1.6 | |
| .30 | 113.9 ± 2.1 | 122.1 ± 2.4 | | 3.7 ±.2 | 3.7 ±.3 | | 31.3 ± 2.2 | 33.1 ± 1.0 | |

The entries represent mean values and 1 stand. error of mean for observations in all 5 men. See legend for Table I.

† $P > .05$. ‡ RP = 1.7 (limits 2.5-1.3). § RP = 1.4 (limits 2.4-.9). ‖ RP = 1.0 (limits 1.4-.7).

changed in the presence of greater distending pressure.

Guanethidine and reserpine deplete tissues of catecholamines rapidly(11-15), but the acute sensitization to norepinephrine is observed only after guanethidine(11,16). This may explain partly the differences in the acute hemodynamic effects of the 2 drugs. Intravenous administration of small doses of guanethidine to dogs increases arterial pressure and cardiac output for periods of 15-30 minutes(17,18); reserpine causes little change(19). This action of guanethidine may be ascribed not only to release of catecholamines(14,15,17-22) but also to the potentiation of their effects(11).

The mechanism of this type of hypersensitivity is obscure. It appears that interruption of sympathetic transmission or depletion of catecholamines do not explain the phenomenon(4,11). McCubbin *et al.*(4) found an augmented pressor response to norepinephrine after guanethidine in dogs treated with reserpine and also in those with spinal cord section at $C_6$. Krayer *et al.*(12) described potentiation of the positive chronotropic effect of norepinephrine by guanethidine in heart-lung preparations of dogs treated with reserpine. It appears that guanethidine may have a direct action on the receptors which increases their sensitivity to norepinephrine.

*Summary.* Intravenous guanethidine enhances the pressor effect of norepinephrine. These experiments show that the increased pressor response in dogs resulted from augmentation of the vasoconstrictor action of norepinephrine rather than from higher blood flow. In humans the increased pressor response was the result of both increased vascular tone and higher flow.

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