(Group A), it is apparent that any ocular defects that may have been present in rats in this group were not sufficiently marked to impair their learning the discrimination problem. The slightly poorer performance of rats in Group B during the early phase of learning (Fig. 1) as compared to that of rats in Groups A and D may have been due to such defects. Additional evidence that the learning performance of rats in Group B was not genuinely lower than that of Group D is indicated by the error score of rats in the 2 groups on day 10 (Table I).

Present findings indicate that prenatal x-irradiation significantly altered behavioral performance of rats exposed at 6 months of age to a brightness discrimination problem. The effects obtained were dependent on stage of pregnancy when x-irradiation was administered and dosage employed. After a single dose of 150 r, discrimination learning performance was impaired only in offspring of rats irradiated on 14th day of pregnancy. After a dose of 300 r, discrimination learning performance was impaired in offspring of rats irradiated on 18th day of pregnancy as well. These observations are in accord with previous findings that prenatal x-irradiation impaired the maze learning performance of rats (2,4,5) and provide additional evidence that sublethal doses of x-irradiation during the latter trimester of pregnancy may result in a learning decrement in the rat.

*Summary.* Offspring of non-irradiated rats which received a single dose of 150 r x-irradiation on 10th, 14th or 18th day of pregnancy respectively or 300 r on 18th day of pregnancy were exposed at approximately 6 months of age to a brightness discrimination problem. Data were evaluated on the basis of number of trials to criterion and number of errors for the various groups on the day when offspring of non-irradiated group reached criterion. Findings indicate that a single exposure of 150 r on 14th day or 300 r on 18th day of pregnancy significantly impaired discrimination learning performance as compared to the performance of non-irradiated rats or offspring of rats administered a single dose of 150 r x-irradiation on 10th or 18th day of pregnancy.

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## Inhibition of Adrenal Medullary Responses to Chemical Stimulation.* (24531)

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Hexamethonium antagonizes the ganglionic stimulation produced by acetylcholine and other depolarizing substances such as nicotine or tetramethylammonium(1). The adrenal medulla is in many ways analagous to a sympathetic ganglion and is known to respond to ganglionic stimulating agents by release of medullary hormones(2). However, the influence of ganglionic blocking agents on responsiveness of adrenals to direct chemical stimulation has not been adequately described. In this study, experiments have been carried out to determine effects of hexamethonium and of

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a newly synthesized substituted urea, N-N-diisopropyl - N'-n-butyl-N'-diethylaminoethyl urea (P-275, Pitman-Moore‡), on responsiveness to the well known chromaffin cell stimulants, nicotine and potassium.

*Method.* Injection of small quantities of nicotine or potassium chloride into the thoracic aorta has been shown previously to produce marked increments in circulatory catechol amines(3) and in cardiovascular functions(4). These responses are thought to be due to direct stimulation of the adrenal medullae since they are not obtained by intravenous injection of equal doses of these agents or by intra-aortic injection in adrenalectomized animals(4). Adrenal stimulation was evaluated in 2 ways: a) by auto-assay technic consisting of recording changes in heart contractile force and arterial blood pressure; b) by fluorimetric determinations of concentrations of epinephrine and norepinephrine in peripheral blood plasma. Experiments were carried out in 14 open-chest, bilaterally vagotomized dogs anesthetised with intravenous sodium pentobarbital (30 mg kg). The chest was opened by mid-line incision and a strain gauge arch(5,6) sutured to right ventricular muscle for measurement of heart contractile force. Blood pressure was obtained with a Statham transducer from a catheter in left femoral artery. This catheter was also used to obtain blood samples for the plasma catechol amine determinations, using a modification(7) of method described by Weil-Malherbe and Bone(8). Intra-aortic injections were made by means of a polyethylene catheter introduced through right femoral artery, into the thoracic aorta, to level of apex of the heart.

*Results.* Fig. 1 shows the typical marked contractile force increments produced by intra-aortic injections of 10 $\mu$g/kg of nicotine and 2 mg/kg of potassium chloride. After administration of 5 mg/kg of hexamethonium intravenously, the heart force response to nicotine is substantially less than the control response, while the effects of potassium are essentially unchanged (Fig. 1).

Table I summarizes mean percentage

‡ Supplied through courtesy of Pitman-Moore Co., Indianapolis, Ind.

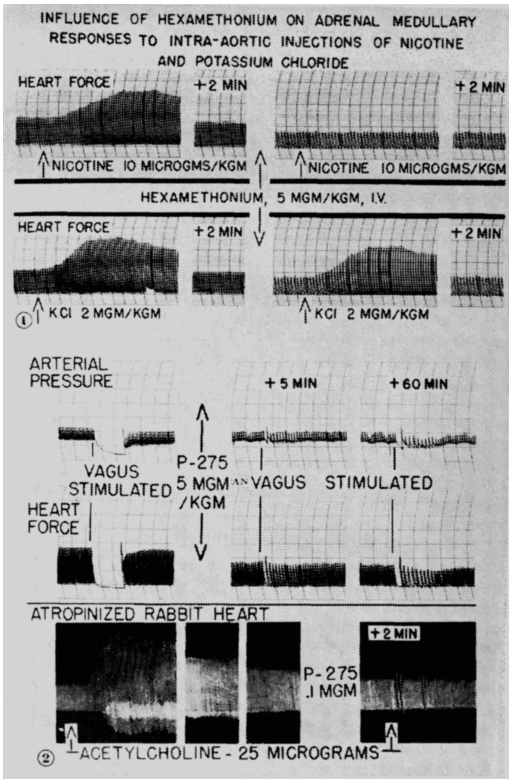


FIG. 1.

changes in heart force and blood pressure obtained in 5 similar experiments, and presents simultaneous variations in plasma levels of epinephrine and norepinephrine. Epinephrine and norepinephrine are presented as mean increases above control values, expressed in $\mu$g/liter of plasma. With nicotine, increments in heart force, blood pressure and plasma epinephrine obtained in the control, or pre-block animals were not obtained after hexamethonium (Table I). In contrast to the lack of responses to nicotine after hexamethonium, the mean responses to potassium appeared to be augmented by administration of the ganglionic blocking agent (Table I). These differences were not statistically significant ($P > .10$).

The effects of compound P-275 on adrenal responsiveness to nicotine and potassium were qualitatively similar to those produced by hexamethonium. Intravenous injection of 5 mg/kg of P-275 substantially reduced the increments in contractile force, blood pressure

TABLE I. Influence of Hexamethonium and P-275 on Adrenal Stimulations Produced by Nicotine and Potassium.

| | Nicotine, 10 μg/kg | | | Potassium chloride, 2 mg/kg | | |
|---|---|---|---|---|---|---|
| | Pre-block | After hexamethonium | After P-275 | Pre-block | After hexamethonium | After P-275 |
| No. observations | 14 (14)† | 5 (5) | 11 (8) | 14 (12) | 5 (5) | 10 (6) |
| | Maximum % increase above control (mean ± S.E.) | | | | | |
| Heart force | 140 ± 15 | 0 ± 0* | 57 ± 19* | 125 ± 17 | 161 ± 67 | 103 ± 22 |
| Blood pressure | 57 ± 11 | 5 ± 3* | 13 ± 5* | 40 ± 4 | 80 ± 23 | 24 ± 8 |
| | Increase above control in μg/l of plasma (mean ± S.E.) | | | | | |
| Epinephrine | 15.9 ± 3 | .2 ± .2* | 3.0* | 12.0 ± 3.9 | 23.0 ± 8.0 | 8.3 ± 2.1 |
| Norepinephrine | 2.1 ± 1 | .2 ± .4 | .5 | 4.0 ± 1.9 | 5.8 ± 1.7 | 1.9 ± .4 |

* Significantly different from Pre-block responses ($P < .05$). † No. of dogs in parentheses.

and plasma epinephrine produced by nicotine, but did not alter responsiveness to potassium (Table I).

The basal levels of catechol amines were not significantly influenced by hexamethonium or P-275. The following are the mean values (± SE) for epinephrine and norepinephrine respectively, expressed as μg/liter of plasma: a) before ganglionic blockade, 0.7 ± 0.2 and 1.7 ± 0.3; b) after hexamethonium, 0.4 ± 0.1 and 2.0 ± 0.5; c) after P-275, 0.6 ± 0.1 and 1.4 ± 0.3.

In addition to its adrenal blocking activity, P-275 was found to have other properties in common with hexamethonium. In 4 experiments, arterial pressure and heart force were recorded in dogs during electrical stimulation of the peripheral end of cut vagus nerve. In the untreated control, vagal stimulation of 5 seconds duration produced cardiac standstill (Fig. 2). Approximately 5 minutes after administration of P-275 (5 mg/kg) similar stimulation provoked only a minimal decrease in heart rate. After one hour, brief cardiac arrest could again be produced, and generally about 2 hours was required before a normal response could again be obtained. P-275 did not alter responsiveness to injected acetylcholine, indicating that the blockade must occur at the ganglionic synapse.

The lower tracing of Fig. 2 illustrates the stimulant effect of a large dose of acetylcholine on the isolated atropinized rabbit heart. Addition of 0.1 mg of P-275 to the perfusion solution completely abolished the response to a subsequent dose of acetylcholine. In several experiments, doses of acetylcholine as high as 200 μg were administered after P-275 and failed to produce any evidence of stimulation. Both of these actions of P-275, namely, vagal ganglionic inhibition and blockade of the acetylcholine response in the rabbit heart, were also produced by comparable doses of hexamethonium.

*Discussion.* Our experiments demonstrate that hexamethonium markedly reduces responsiveness of the adrenal medulla to direct stimulation with nicotine, while the effects of potassium are unchanged. Previous investigators have shown that the ganglionic excitant effects of potassium are not significantly altered by tetraethylammonium(9). These data indicate that the adrenal medulla is identical to other sympathetic ganglia in its reaction to ganglionic blocking agents(1,9). Thus it would appear that the ganglionic blocking activity of drugs could be simply and effectively estimated by determining their ability to antagonize adrenal responsiveness to direct chemical stimulation. The experiments with compound P-275 support this hypothesis.

P-275 is a newly synthesized substitute of urea which has been shown by its commercial sponsors to abolish the pressor response to large doses of acetylcholine in atropinized dogs, and to reduce the hypertensive effect of splanchnic nerve stimulation (personal communication). In the present experiments, this compound was found to be similar to hexamethonium in reducing the adrenal excitant effects of nicotine, but not of potassium. Further evidence of the ganglionic blocking activity was obtained in demonstrating that P-275, in common with hexamethonium, pro-

duced vagal ganglionic inhibition and blockade of the stimulant effect of acetylcholine on the isolated rabbit heart.

*Summary.* Hexamethonium reduced sensitivity of adrenal medulla to nicotine, but not to potassium. A newly synthesized compound. N-N-diisopropyl-N′-n-butyl-N′-diethylaminoethyl urea (P-275) was similar to hexamethonium in its effects on the adrenal, as well as in its ability to inhibit the cardiovascular depressant effects of vagal stimulation and the acetylcholine stimulation of the isolated atropinized rabbit heart.

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## Isolation of Mucosal Disease Virus by Tissue Cultures in Mixture 199, Morgan, Morton and Parker.* (24532)

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Isolation of viruses by tissue culture methods from cattle with bovine mucosal disease. infectious bovine rhinotracheitis, and virus diarrhea of New York type of mucosal disease complex(1) have been reported by at least 4 teams of investigators. Underdahl, *et al.*,(2) isolated a cytopathogenic virus from cattle having bovine mucosal disease (BMD). York. Schwartz and Estela(3) and Madin and his associates(4) isolated viral agents from cattle having infectious bovine rhinotracheitis; Lee and Gillespie(5) isolated a strain of virus from cattle with virus diarrhea New York. All foregoing virus isolations employed trypsinized cells and medium containing serum from cattle, horse or lamb. Lee and Gillespie also used roller tube explant method isolation procedures. Underdahl (personal communication) believed that his success in isolation and cultivation of a viral agent from animals having BMD depended upon using a homologous system of bovine kidney cells and bovine serum. He obtained antibody free serum by drawing blood from a newborn calf which had not been allowed to nurse. However, Schipper and Eveleth(6) report calves being born and dying 18 to 96 hours after birth showing pathology typical to BMD upon necropsy. They also report similar pathology in calves born dead at full term. In one instance, necropsy of a mature cow diagnosed as having mucosal disease possessed an 8½ month old fetus with typical lesions of mucosal disease. No BMD virus or hyperimmune serum was demonstrated from such calves since virus isolations procedures were not yet established. Since that time however, we have had a still born full term calf which showed pathology typical to BMD. While no viruses were recovered from biopsies of this calf, the serum, when diluted 1:64, was capable of neutralizing 1000

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