results indicate that following a severe insult, increased taurine is excreted that is derived from a tissue pool. An alternate theory to that previously mentioned, the shift of taurine metabolic pathways, could be that deoxypyridoxine results in a release of tissue taurine stores with subsequent urinary excretion.

Summary. Deoxypyridoxine, a vitamin B_6 antagonist, was administered to 4 patients with Downs' syndrome and controls matched for age and weight. Taurine excretion was measured in 24-hour urine samples collected before and during the period of deoxypyridoxine administration. Taurine excretion in the Downs' syndrome patients before deoxypyridoxine was significantly less than their controls ($p<0.01$). During deoxypyridoxine administration, average taurine excretion of both groups increased on Day 1, decreased on Day 2, then increased significantly above base line levels by Day 10. There was no significant difference in taurine excretion between Downs' syndrome patients and their controls. Trend analysis for a quadratic component showed that the decrease and subsequent increased excretion of taurine was of statistical significance. Possible reasons for the observed changes were discussed. Taurine excretion during deoxypyridoxine administration differs from that found during vit B_6 depletion in man.

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Influence of Propranolol on Hemodynamic Changes and Plasma Catecholamine Levels Following Cigarette Smoking and Nicotine.* (31434)

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It is well known that cigarette smoking and nicotine administration stimulate man's cardiovascular system(1). Among the effects commonly seen are an increase in blood pressure, heart rate, stroke volume and cardiac output(2-4). Many of these effects can be attributed to the release of catecholamines from the adrenal medulla and adrenergic nerve endings as observed by Westfall and Watts(5-7) and Kershbaum *et al*(8,9).

At present it is popular to attribute the pharmacological action of catecholamines to their combination with certain receptors. One such classification of adrenergic receptors is that of Ahlquist(10) who arbitrarily chose the names of *alpha* and *beta* based on the relative

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effectiveness of various agonists in inducing a series of selected responses. It is thought that the *alpha* receptors are mainly responsible for sympathetic excitatory functions except for the inhibitory effect on the intestine while the *beta* receptors are concerned predominantly with inhibitory functions with the important exception of the excitatory effect on the heart (both positive inotropic and chronotropic effect). The *alpha* receptors can be selectively blocked by such antagonists as phenoxybenzamine, ergot alkaloids and phentolamine(11-13) while the *beta* receptors can be specifically blocked by agents only recently available such as dichloroisoproterenol, pronethalol and propranolol(14-16).

The purpose of this present investigation was to study the effect of one of these newer *beta* adrenergic antagonists, propranolol (1-isopropylamine-3-(1-naphthyloxy)-3-propanol hydrochloride), on the hemodynamic and catecholamine releasing properties of cigarette smoke inhalation and nicotine administration in the dog.

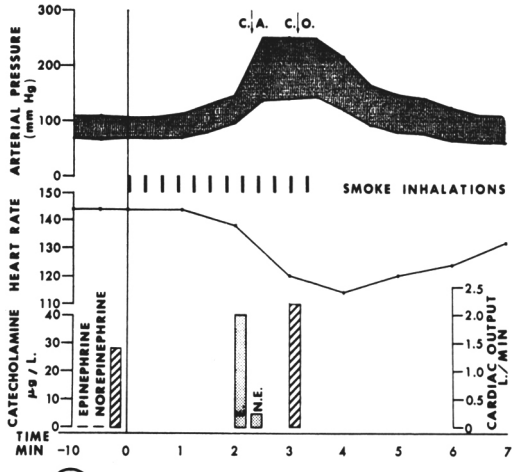
Methods. Experiments were carried out on mongrel dogs of either sex with weights ranging from 15-25 kg. Following preanesthetic medication with morphine (5 mg/kg) the dogs were anesthetized with pentobarbital sodium (25 mg/kg, intravenously). Number 6 cardiac catheters were positioned in the superior vena cava and pulmonary artery *via* the jugular veins and the high inferior vena cava and low inferior vena cava *via* the femoral veins. An indwelling cannula was also inserted into a femoral artery connected with polyethylene tubing and a Danish 3-way stopcock to (1) a Statham P23D strain gauge, (2) an open end for sampling blood and (3) a Gilford densitometer and Harvard infusion withdrawal pump. Heart rate, arterial blood pressure and electrocardiogram (ECG) were recorded by means of a C.E.C. ultraviolet oscillograph. Cardiac output was determined by the indicator dilution technique using Indocyanine green. A cuffed endotracheal tube was inserted and connected to a standard respirometer for inhalation of cigarette smoke.

For catecholamine determinations 20 ml blood samples were simultaneously obtained from the high and low inferior vena cava and immediately placed in iced centrifuge tubes containing disodium ethylenediaminetetraacetic acid (EDTA) (20 mg) and sodium metabisulfite (0.5 mg/ml). To prevent sympathoadrenal stimulation as a result of blood loss an equal volume of blood from a donor dog was promptly replaced into the experimental animal. The collected blood samples were immediately centrifuged at 3,000 $\times$ *g* for 25 minutes in a refrigerated centrifuge. The plasma was removed and an equal volume of 0.2 M sodium acetate added. The pH of the sample was adjusted to 8.3 with 0.5 M sodium carbonate, the amines absorbed on alumina and eluted with 0.2 acetic acid. Following elution, epinephrine and norepinephrine determinations were carried out on a Technicon autoanalyzer according to the automated trihydroxyindole method of Robinson and Watts(17).

Control measurements of blood pressure, heart rate and cardiac output were obtained with simultaneous high and low caval blood samples for catecholamine determinations. The animals were then made to inhale smoke from a standard unfiltered brand of cigarette *via* a holder placed on the respirometer. The rate and depth of cigarette smoke inhalation was controlled by a clamp placed on the side arm of the cigarette holder and was kept constant through all the experiments. The normal rate was one smoke inhalation every third inspiration over a 3-minute period. The blood pressure, heart rate and ECG were continuously monitored. Blood samples for catecholamine determination and cardiac output measurements were made during the peak of the blood pressure response (Fig. 1 and 2).

After 30-60 minutes, post smoking measurements were obtained. Similar measurements were made following intravenous infusion of 50 μg/kg nicotine pure base diluted in saline and administered over a one-minute period. This dose of nicotine is thought to be within the range systemically absorbed as a result of cigarette smoke inhalation(18).

After observations of the effects of cigarette smoke inhalation and nicotine administration were completed, *beta* adrenergic blockade was produced by administration of 0.2 mg/kg propranolol, intravenously. The degree of



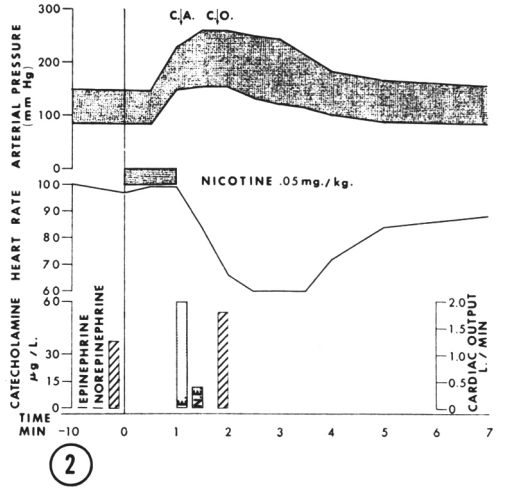


FIG. 1. Representative experiment of effect of cigarette smoke inhalation on arterial blood pressure in mm Hg (top) heart rate in beats per min (middle) vena cava epinephrine and norepinephrine levels in μg/l (bottom, stippled bar) and cardiac output in l/min (bottom, diagonal bars) in the dog. Markings under arterial pressure represent smoke inhalations. Numbers on abscissa represent time in min. Arrows at CA and CO represent time of sampling for catecholamine analysis or cardiac output determination respectively.

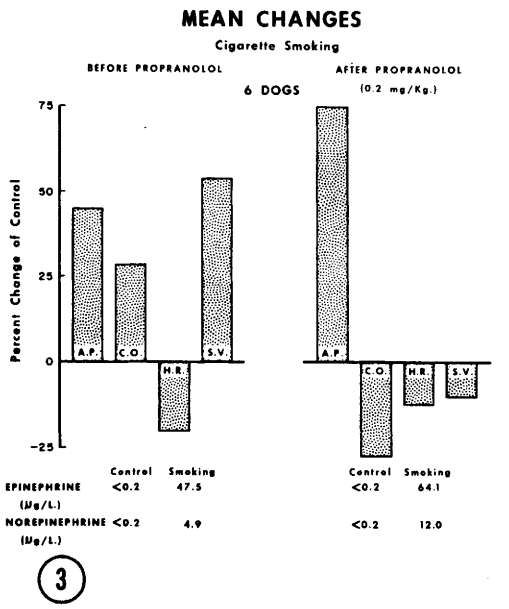
FIG. 2. Representative experiment of effect of nicotine infusion on the same parameters as Fig. 1. Smoke inhalations have been replaced with a one min infusion of nicotine (0.05 mg/kg) intravenously.

blockade was ascertained in each dog by determining the amount of antagonism produced against the positive chronotropic effect of infusions of isoproterenol (1.25 to 2.5 μg/min). This dose of propranolol produced 75-80% block. After this degree of blockade was produced the cardiovascular response to cigarette smoke inhalation and nicotine administration was again studied and the results compared with the responses observed before blockade. Statistical analysis of the data was carried out according to student's t test.

Results. A representative experiment of the effect of cigarette smoke inhalation on arterial blood pressure, heart rate, cardiac output and central venous catecholamine content is shown in Fig. 1. This dog had a total of 12 smoke inhalations per 3-minute period. By the second minute there was a marked increase in systolic and diastolic pressure and reflex slowing of the heart rate. There was also a marked increase in the plasma catecholamine level as well as in cardiac output. Several minutes after the cessation of smoking, all measurements had returned to normal.

Fig. 2 shows a representative experiment in a different dog of the effect of an infusion of 50 μg/kg of nicotine. Although the nicotine was given at a faster rate than the smoke inhalations, the pattern is similar.

Mean changes in arterial pressure, cardiac output, heart rate, stroke volume and high vena cava epinephrine and norepinephrine content following cigarette smoke inhalation before and after *beta* adrenergic blockade with propranolol are shown in Fig. 3. Before propranolol, cigarette smoke inhalation produced increases of 45, 27 and 55% from control in arterial blood pressure, cardiac output and stroke volume respectively. The heart rate decreased 22% from control. The epinephrine level in the high vena cava blood increased from <0.2 to 48 μg/l and the norepinephrine level from <0.2 to 5 μg/l. All these values were significantly different from control ($P<0.05$). Following *beta* adrenergic blockade there was a greater increase in arterial pressure, to 75% above control. This response was significantly greater than that before blockade ($P<.001$). Cardiac output and stroke volume were decreased by 27 and 11% from control levels respectively; both differed significantly from the response observed before blockade ($P<.001$). The decrease in heart rate was less (—12% from



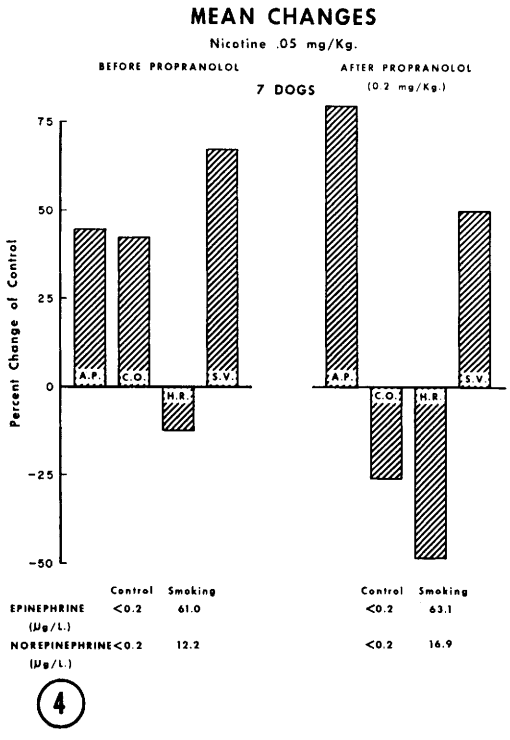


FIG. 3. Summary of effect of smoke inhalation on mean arterial blood pressure (A.P.) cardiac output (C.O.), heart (H.R.) and stroke volume (S.V.) before and after *beta* adrenergic blockade with propranolol (0.2 mg/kg, intravenously). All values represent per cent change from control. High vena cava plasma epinephrine and norepinephrine levels are also included in μg/l. Values represent mean changes from 6 dogs.

FIG. 4. Summary of effect of nicotine infusion (0.05 mg/kg during 1 min) on same parameter as Fig. 3. Value represent mean changes from 7 dogs.

control) and was not statistically different. The increases in epinephrine, from <0.2 to 64 μg/l and norepinephrine, from <0.2 to 12 μg/l, were not statistically different from the response prior to blockade with propranolol.

Fig. 4 summarizes the effect of a one-minute infusion of 50 μg/kg of nicotine on hemodynamic responses and catecholamine levels before and following *beta* adrenergic blockade. Prior to blockade with propranolol, the nicotine infusion produced increases of 46% in arterial pressure, 43% in cardiac output and 68% in stroke volume, and a 13% decrease in heart rate. In addition there was an increase in the high inferior vena cava epinephrine levels from <0.2 to 61 μg/l and in norepinephrine from <0.2 to 12 μg/l. During blockade 15-30 minutes prior to nicotine administration, there was an increase of 80% in arterial pressure while the cardiac output was decreased 26%. Propranolol administration reduced the increase in stroke volume to 48% while the heart rate decreased 47% from the controls. All these values were statistically different from those prior to blockade ($P<.05$). There was an increase in the high vena cava epinephrine level from <0.2 to 63 μg/l and in norepinephrine from <0.2 to 17 μg/l. These responses were not statistically different from those before blockade.

Discussion. These experiments in intact anesthetized dogs are consistent with previous observations of the stimulatory effect of cigarette smoke inhalation or nicotine administration on the cardiovascular system(1-4) and on adrenal gland catecholamine release(5-9). They also demonstrate marked alterations in the above cardiovascular responses during *beta* adrenergic blockade.

The increase in mean arterial blood pressure was much greater in response to smoking and nicotine administration following propranolol than before blockade. This would suggest that, by producing blockade of *beta* adrenergic receptors, more *alpha* receptors are made available or "unmasked." Thus, the

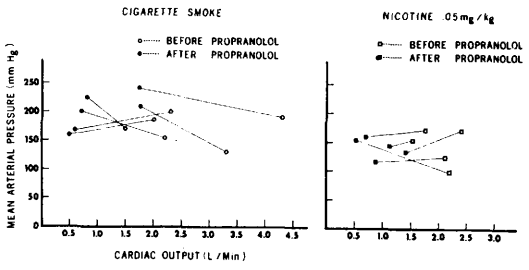


FIG. 5. Effect of cigarette smoke inhalation and nicotine administration on systemic resistance as obtained by plotting mean arterial pressure against cardiac output before and after *beta* adrenergic blockade with propranolol (0.2 mg/kg, intravenously).

vasoconstriction produced by the action of epinephrine on *alpha* receptors is unopposed by vasodilation *via beta* receptors in such vascular beds as skeletal muscle. Thus there was a great increase in systemic peripheral resistance resulting from cigarette smoke inhalation and nicotine infusion during *beta* adrenergic blockade. This was confirmed by plotting the changes in mean arterial pressure against the changes in cardiac output in individual dogs (Fig. 5).

The marked decrease of cardiac output and stroke volume following cigarette smoke inhalation or nicotine infusion during *beta* adrenergic blockade suggests a lessened inotropic effect on the left ventricular myocardium during this period of increased peripheral vascular resistance. This was accompanied by increases in pulmonary wedge pressure and in several instances overt left ventricular failure. These hemodynamic changes were not due to a greater increase in circulating catecholamines since there was no statistically significant difference in the amount of catecholamines released as a result of these procedures before or during *beta* adrenergic blockade.

These findings are consistent with those of Harris *et al*(19) who studied the effect of *beta* adrenergic blockade with propranolol on the hemodynamic responses to epinephrine and isoproterenol in man. The heart rate, stroke volume and cardiac output responses changed from increases to decreases, while there was a greater increase in mean arterial pressure and total peripheral resistance. Harris *et al*(19) also concluded that the hemodynamic responses to epinephrine after propranolol did not result from changes in body metabolic rate since in supine subjects oxygen consumption was unaffected either by 10 mg of propranolol intravenously, or by 5 μg/min of epinephrine intravenously after propranolol.

The present experiments are also consistent with those of Shanks(20) who also observed a greater blood pressure response to epinephrine following *beta* adrenergic blockade with propranolol in dogs as well as Marshall *et al*(21) who observed a considerable increase in blood pressure, relative bradycardia and a consistent decrease in cardiac output to epinephrine following propranolol blockade in dogs.

Summary. Cigarette smoke inhalation or intravenous infusion of nicotine produced a marked increase in cardiac output, stroke volume, arterial blood pressure and plasma epinephrine and norepinephrine levels with a reflex decrease in heart rate in the morphine-pentobarbital anesthetized dog. Following *beta* adrenergic blockade with 0.2 mg/kg propranolol intravenously, cigarette smoke inhalation and nicotine infusion resulted in a marked alteration of cardiac output, stroke volume, heart rate and mean arterial pressure without significant changes in caval plasma catecholamine levels. It is concluded that *beta* adrenergic blockade produced by propranolol does not modify the release of catecholamines from the adrenal glands of dogs in response to smoking and nicotine and that the "unmasking" of *alpha* receptor activity causes a marked increase in systemic peripheral resistance and compromises left ventricular function.

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Influence of Antigens on Release of Free Fatty Acids from Arlacel A (Mannide Monooleate). (31435)

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A high incidence of sterile abscess formation in women vaccinated with mineral oil and 7-n-hexyloctadecane emulsified tetanus toxoids from one of two series of preparations was reported by MacLennan *et al*(1). Abscess formation following use of emulsified cholera vaccine(2) and emulsified typhoid vaccine(3) also has been reported. We undertook the present studies to find the cause of these reactions.

Mannide monooleate (Arlacel A, Atlas Chemical Industries) has been used extensively as an emulsifier in the preparation of vaccines and repository allergens for experimental use in man(1-8). Sterile abscess formation observed in recipients of water-in-oil emulsions of influenza virus vaccine(4,9-11) was attributed to the use of impure batches of Arlacel A(4) which had a high content of free oleic acid(11). Subsequently, the lots of Arlacel A to be incorporated in vaccines were tested for freedom from toxicity by the Berlin criteria(12). Nevertheless, adverse conditions of storage and transportation might cause hydrolysis of the ester linkage of the mannide monooleate(12,13) with release of oleic acid prior to the use of the emulsifier. In addition, substances in the antigen might cause the release of free fatty acids in the final biologic product.

The present report shows that the emulsified toxoids and the cholera and the typhoid vaccines which caused sterile abscesses contained free fatty acid (FFA) and that a variety of antigens is capable of releasing free fatty acid from a water emulsion of Arlacel A. This report also contains preliminary data on the characterization of an active substance in *Clostridium tetani* culture filtrate which hydrolyzed Arlacel A.

Materials and methods. Antigens. Tetanus toxoids B_1, C_1, D_1, A_2, B_2, C_2 and D_2 were kindly prepared by Parke, Davis & Co. for use in the field study in New Guinea(1). The toxoids with subscript 1 were prepared from a common lot of toxoid containing 612 Lf/mg total nitrogen (TN) while toxoids with subscript 2 were prepared from another lot of