lated to the rapidly increasing concentration of cocaine in plasma and not to peak values. Alternatively, since cocaine is highly lipid soluble, the euphoria may better correlate with concentrations at brain receptor sites than with peak concentrations in plasma. Nayak *et al.* (*19*) found significant amounts of cocaine and its active metabolite, norcocaine, in the brains of rats after intravenous (8 mg per kilogram of body weight) and subcutaneous (20 mg per kilogram of body weight) injections.

The development of a toxic psychosis in those individuals who repeat the dosage every 15 minutes might be explained by accumulation secondary to the persistence of cocaine in the plasma for 4 to 6 hours. Since cocaine plasma concentrations may remain elevated during surgical procedures, it might also potentiate sympathomimetic amines given later in the course of such procedures. This possibility should be considered in the clinical use of cocaine.

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6. Seven of the CV patients had coronary artery bypass grafts while one patient had a mitral valve replacement and one patient had both a coronary artery bypass graft and an aortic valve replacement.
7. Three of the dental patients had extractions of their third molars while one dental patient had the excision of a small benign intraoral lesion.
8. We have since determined that sodium fluoride (0.13 percent) in plasma will completely inhibit the in vitro hydrolysis of cocaine for 2 hours at 37°C and for at least 6 weeks at −15°C.
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10. The internal standard was synthesized by overnight refluxing of benzoylecgonine in acidified *n*-propanol, and purified by preparative thin-layer chromatography.
11. A Perkin-Elmer model 3920 gas chromatograph (GC) with a separately heated rubidium glass nitrogen detector was used. The GC conditions were as follows: oven temperature, 260°C, isothermal; He flow rate, 20 ml/min; column, 3 percent OV-17 on 100–120 mesh Gas Chrom Q.
12. Arterial blood samples were obtained from CV patients and venous blood samples were obtained from dental patients. To ascertain whether this affected our results, we compared simultaneous arterial and venous blood samples in CV patients on two occasions (a 3-minute sample and a 60-minute sample) and found a venous-arterial plasma level ratio of 0.84 and 0.98, respectively. Other investigators [E. Erikssen, S. Englessen, S. Wahlquist, B. Ortengren, *Acta Chir. Scand. Suppl.* **358**, 25 (1966)] found that the peripheral venous-arterial blood concentration ratio for lidocaine was 0.73 ± 0.03 (mean ± S.E.) and for prilocaine was 0.47 ± 0.03. They attributed this difference to the rapid uptake of these local anesthetics by skeletal muscle.
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17. They used a colorimetric method 50 to 100 times less sensitive than our procedure and were able to detect minimum values of 500 to 1000 ng/ml [L. A. Woods, J. Cochin, E. J. Fornefeld, F. G. McMahon, M. H. Seevers, *J. Pharmacol. Exp. Ther.* **101**, 188 (1951); L. A. Woods, F. G. McMahon, M. H. Seevers, *ibid.*, p. 200].
18. The swabs were eluted with 0.1*N* H_2SO_4. The aqueous phase was adjusted to *p*H 9.6 to 9.8 with carbonate buffer, and extracted with ether. The ether was evaporated and the residue was analyzed by the use of TLC with development in ethyl acetate, methanol, and ammonia (85 : 10 : 5) and sprayed with potassium iodoplatinate.
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Nicotinic Acid Reduction of Plasma Volume Loss After Thermal Trauma

Abstract. *Intravenous administration of nicotinic acid to the anesthetized dog prior to thermal trauma reduced plasma loss at 10 minutes after burn from 7 milliliters per kilogram to less than 2 millimeters per kilogram. During the next 50 minutes plasma loss was the same in treated and untreated animals. An additional dose of nicotinic acid 30 minutes after burn prevented this further loss.*

Increased vascular permeability and plasma volume loss occur rapidly after thermal injury to either humans or animals. Although the loss of plasma was recognized early (*1*), systematic studies of this plasma extravasation are relatively recent (*2*). The physiological mechanisms responsible for this alteration of vascular permeability are not known at present. One or more mediator substances may be released directly as the result of the trauma or indirectly by the activation of the sympathetic nervous system (*3*). In order to test this postulate, we examined a number of pharmacological agents to determine whether it was possible to reduce the loss of plasma volume after thermal injury to experimental animals. The agent which has been most interesting to us has been nicotinic acid.

Mongrel dogs of either sex, weighing between 8 and 15 kilograms, anesthetized with sodium pentobarbital (30 to 35 mg per kilogram of body weight) were used in these studies. Isotopically labeled (^{125}I) albumin was injected into the femoral vein. Arterial samples were collected at timed intervals after injection. The plasma volume at the time of injection was determined by retrograde extrapolation. Plasma volumes were determined prior to burn and at 10 minutes and at 60 minutes after trauma. Three groups of experimental animals were studied. The first and second groups of animals were studied on alternate days and the third group of animals was studied immediately upon completion of study of the first two groups. All animals received a third-degree flame burn over 40 percent of their body area. One

Table 1. Plasma volume loss in mongrel dogs after flame burn. Eight animals received no treatment, six animals received nicotinic acid (10 mg/kg) before burn, and eight animals received nicotinic acid (10 mg/kg) before burn and an additional dose (5 mg/kg) after burn.

Treatment	Plasma volume loss (ml/kg)		
	0 to 10 min after burn	10 to 60 min after burn	0 to 60 min after burn
None	7.11 ± 1.47	4.99 ± 1.87	12.10 ± 1.73
Nicotinic acid*	0.89 ± 1.01†	5.94 ± 1.43	6.83 ± 1.95†
Nicotinic acid‡	1.88 ± 0.41†	0.52 ± 0.71†§	2.40 ± 0.52†§

*10 mg/kg before burn. †Indicates significant difference by unpaired *t*-test from untreated group ($P < .05$). ‡10 mg/kg before burn and 5 mg/kg 30 minutes after burn. §Indicates significant difference by unpaired *t*-test from group receiving 10 mg/kg before burn.

group of animals served as a control and received no treatment following the burn. The second group of animals received 10 mg of nicotinic acid (Lilly N.F.) per kilogram of body weight intravenously before burn and immediately after the initial plasma volume determination. The third group of animals received the same dose of nicotinic acid with an additional dose of 5 mg of nicotinic acid per kilogram of body weight administered approximately 30 minutes after the burn (Table 1).

These experiments suggest that nicotinic acid administered prior to burn reduces plasma loss associated with burns. In addition, the duration of action of this substance is approximately 1 hour. The administration of additional maintenance doses of nicotinic acid appears to minimize plasma loss for an extended period. The mechanism of action of nicotinic acid is not known but it may involve the prostaglandins.

Nicotinic acid in large doses will prevent catecholamine-induced release of free fatty acids (*4*). Arachidonic acid is a precursor substance for the formation of the prostaglandins E_2 and $F_2\alpha$ (*5*). Intravenous arachidonic acid will cause rapid platelet clumping and sudden death; these effects may be prevented by the administration of aspirin, an inhibitor of prostaglandin synthetase (*6*). The administration of another inhibitor of prostaglandin synthetase, indomethacin, will significantly reduce the loss of plasma volume in dysbaric dogs (*7*). Based upon these observations, we postulate that nicotinic acid in minimizing the plasma loss due to thermal injury involves inhibition of the release of fatty acids induced by catecholamine. This should diminish concentration of the probable precursor (arachidonic acid) for prostaglandin synthesis.

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Estrogen Receptor in the Mammalian Liver

Abstract. *The cytosol from livers of adult female mammals contains [^{3}H]estradiol-binding proteins that can translocate to the nucleus and attach to chromatin. In comparison to the prepubescent rat, adults have higher estrogen binding in the liver and greater increases in plasma renin substrate after administration of estrogen. The protein in the liver which binds estrogen may be an estrogen receptor involved in modulating hepatic synthesis of selective plasma proteins.*

Estrogens and other steroid hormones appear to initiate their action by binding with intracellular cytosol receptor proteins (*1*). Hormone action then proceeds by the translocation of the steroid receptor complex to the nucleus (*1*). Binding with a high affinity and specificity for estrogen has not been found previously in the liver supernatant of adult mammals (*2*), although administration of synthetic estrogens causes striking increases in plasma proteins of hepatic origin (*3*) and produces biochemical changes in the liver (*4*). We have found that the supernatant fraction of adult mammalian livers contains macromolecules that bind [^{3}H]estradiol with high affinity and specificity. After estradiol interacts with the binding protein the complex appears to be able to translocate to the nucleus and attach to chromatin. In the rat, the development of this binding with sexual maturation correlates with the ability of the administration of high doses of estrogen to increase the concentration of the plasma protein, renin substrate. We postulate that the estrogen-binding macromolecule in the liver supernatant is the estrogen receptor and that it modulates hepatic synthesis of plasma proteins that may be involved in mediating serious side effects of estrogen-containing contraceptives.

Livers of adult female Charles River CD rats, or other animals as indicated, were homogenized in six volumes of 0.01*M* tris-HCl (*p*H 7.4) with 0.0015*M* ethylenediaminetetraacetate (EDTA), and the supernatant fraction was prepared by ultracentrifugation at 100,000*g* for 1 hour. Unless otherwise specified, the reaction conditions were the incubation of $2 \times 10^{-9}M$ radioactive estradiol ([^{3}H]E_2) (100 c/mmole) with 0.2 ml of supernatant for 1 hour in ice. Macromolecular-bound radioactivity was then separated from free radioactivity by small polyacrylamide gel filtration columns (BioGel P10, exclusion molecular weight 20,000). Radioactivity in the macromolecular fraction was then extracted into toluene and assayed by scintillation spectrometry (*5*). Each experimental group consisted of at least triplicates, and all results are reproducible upon repetition.

In adult female rats the binding averages 6200 disintegrations per minute (dpm) in 0.2 ml of liver supernatant (1.0 fmole per milligram of tissue) or 1900 dpm per milligram of supernatant protein (8.7 fmole per milligram of supernatant protein). Six percent of the [^{3}H]E_2 is bound. This is lower than found with the uterus but 4 to 20 times higher than with a nontarget organ, such as the heart, or with plasma. As will be described below, the liver binding can be increased by using experimental conditions developed to study the binding at equilibrium. The radioactivity extracted from the bound fraction has been identified as unchanged estradiol by thin-layer chromatography and by methylation to 3-methoxyestradiol. The binding is highly estrogen specific. Nonradioactive estrogens reduce the binding of [^{3}H]E_2 while androgens, progesterone, and glucocorticoids do not (Table 1). The most effective competitors are the potent estrogens estradiol, 17α-ethinyl estradiol, and diethylstilbestrol (DES). The macromolecules contain protein; the binding is diminished after incubation with proteolytic enzymes. The binding is also diminished in the presence of a reagent, sodium *p*-chloromercuriphenylsulfonate (PCMS), that reacts with sulfhydryl groups. After density gradient

Table 1. Specificity of the liver supernatant [^{3}H]E_2 binding macromolecules. [^{3}H]Estradiol ($2 \times 10^{-9}M$) was mixed with $1 \times 10^{-7}M$ nonradioactive hormones and then added to liver supernatant and incubated for 1 hour at 0°C. The enzymes (0.25 mg/ml) and [^{3}H]E_2 were incubated with liver supernatant at 25°C for 1 hour. The liver supernatant was incubated at 0°C with $5 \times 10^{-3}M$ PCMS for 30 minutes followed by the addition of [^{3}H]E_2 for one more hour. Macromolecular binding of [^{3}H]E_2 was then determined by gel filtration. Results are expressed as the percentage of corresponding control ± standard error of the mean.

Chemicals added to incubation mixture	Percent of control
Hormones	
Estradiol	8.5 ± 0.6*
Ethinyl estradiol	6.6 ± 0.3*
DES	10 ± 2*
Testosterone	110 ± 3
Dihydrotestosterone	109 ± 9
Progesterone	118 ± 5
Corticosterone	109 ± 3
Dexamethasone	111 ± 6
Enzymes	
Papain	6 ± 2*
Trypsin	9 ± 1*
Chymotrypsin	20 ± 1*
Ribonuclease	98 ± 2
Sulfhydryl reacting reagent	
PCMS	16 ± 1*

*$P < .05$, significantly lower than controls.