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.

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.

Livers of adult female Charles River CD rats, or other animals as indicated, were homogenized in six volumes of $0.01M$ tris-HCl (*p*H 7.4) with $0.0015M$ 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 sedimentation through sucrose buffered with 0.01*M* tris-EDTA, the gel-filtered protein sediments in the 4*S* and 8*S* regions (Fig. 1A). When dialysis conditions are used that minimize changes in steroid concentrations due to metabolism during the 24-hour equilibration, the liver supernatant exhibits a high affinity for binding estradiol (Fig. 1B). The equilibrium dissociation constant for binding (to a component that can be inhibited by addition of DES) is $0.7 \times 10^{-10}M$ at 4°C. The capacity of the high affinity system is 4.7 fmole per milligram of tissue (58 fmole per milligram of supernatant protein). The binding protein is partially purified by ammonium sulfate fractionation. The protein is precipitated by ammonium sulfate at 30 percent of saturation, with 20-fold increase in the binding per milligram of protein relative to cytosol.

These properties of the estrogen-binding system of the liver do not correspond to known hepatic enzymes (*6*). The liver supernatant does contain enzymatic activity that will oxidize half of the estradiol to estrone in 1 hour at 22°C. The macromolecular binding proteins with high affinity for estrogens can be distinguished from this enzyme by addition of DES to the incubation mixture. Addition of $10^{-7}M$ nonradioactive DES abolishes the high affinity binding but does not diminish the oxidation.

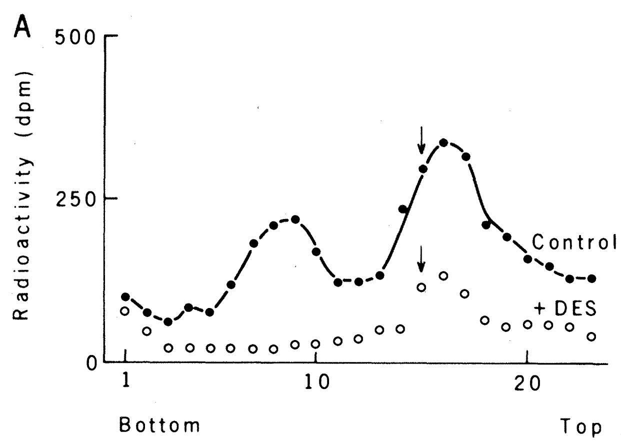
Table 2. Macromolecular binding after incubation of $[^3H]E_2$ with liver slices and with liver chromatin. (A) Liver slices (0.5 to 1 mm thick) from adult female rats were incubated in a Krebs-Ringer phosphate buffer containing $5 \times 10^{-9}M$ $[^3H]E_2$ at 25°C for 1 hour under an atmosphere of 95 percent O_2 and 5 percent CO_2. Slices were washed with fresh ice-cold incubation buffer and homogenized in buffered isotonic sucrose solution. Cytosol was prepared from the homogenate by centrifugation at 1,000*g* for 10 minutes followed by 105,000*g* for 1 hour. Nuclei were prepared by centrifugation of the 1000*g* pellet through 2.2*M* sucrose containing 0.005*M* $MgCl_2$ and Triton X-100 (0.2 percent). (B) Liver supernatant, 0.2 ml (with and without DES), albumin, or rat plasma, each containing 11 mg of protein per milliliter or tris-EDTA buffer, was incubated with $2 \times 10^{-9}M$ $[^3H]E_2$ for 1 hour in ice. Liver chromatin (0.1 ml containing 135 μg of DNA), prepared by freezing and thawing purified nuclei in 0.01*M* tris buffer, was added and the mixture was incubated at 25°C or in ice for 30 minutes. The chromatin was sedimented and washed by resuspension and sedimentation. Nuclei (A) or chromatin (B) were extracted with a mixture of 2*M* NaCl and 5*M* urea for 15 minutes in ice, and macromolecular binding was measured by gel filtration of the extract. Macromolecular binding in the supernatant was also measured by gel filtration. Radioactivity present in the macromolecular-bound fraction was extracted into toluene and counted. In (B) the residual supernatant binding was 14,000 dpm at 25°C and 12,800 dpm at 0°C. Addition of DES decreased supernatant binding to 900 dpm at 25°C. The tabulated results are averages ± standard error of the mean. IT, incubation temperature; LS, liver supernatant.

(A) Liver slices		
IT (°C)	Radioactivity bound (dpm per gram of liver)	
	Supernatant	Nucleus
0	48,000 ± 6,300	1,400 ± 440
25	42,000 ± 5,200	5,800 ± 1,000*

(B) Liver chromatin binding		
IT (°C)	Incubation medium	Radioactivity bound (dpm)
25	LS	3,000 ± 50*
0	LS	200 ± 20
25	LS + 5 $\times 10^{-7}M$ DES	80 ± 20
25	Albumin	90 ± 10
25	Plasma	50 ± 10
25	Buffer	270 ± 110

*Significantly higher nuclear and chromatin binding than in other groups; $P < .05$.

Many of these properties of the hepatic estrogen-binding macromolecule resemble those of cytoplasmic steroid receptors in their target organs, such as the estrogen receptor in the uterus (*1*). In the uterus after estrogen binding to the receptor in the cytoplasm, the entire estrogen receptor complex is thought to translocate to the nucleus in a temperature-dependent process and attach to chromatin (*1*). We incubated radioactive estradiol with slices of liver and then measured macromolecular binding in



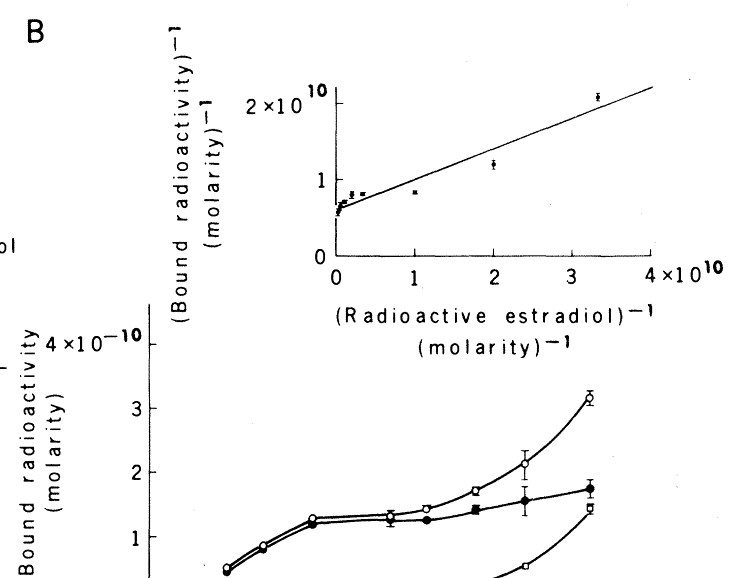


Fig. 1. Properties of the macromolecular binding of $[^3H]E_2$ in rat liver supernatant. (A) Sucrose gradient ultracentrifugation. Liver supernatant was incubated with $5 \times 10^{-9}M$ $[^3H]E_2$ in the presence and absence of $1 \times 10^{-7}M$ DES on ice for 1 hour. The macromolecular-bound radioactive fraction was separated by gel filtration. A portion of the bound fraction corresponding to 0.1 ml of cytosol was layered onto 5 to 20 percent sucrose gradients with 0.01*M* tris-HCl (*p*H 7.4), 0.01*M* KCl, 0.001*M* NaN_3, and 0.0015*M* EDTA. The samples were centrifuged in a Spinco L5-65 in the SW56 Ti rotor at 36,000 rev/min for 16 hours. Eight drop fractions were collected, after puncturing the bottom of the tubes, and counted. The arrows indicate the position of the albumin sedimentation coefficient marker (4.5*S*). (B) Saturation analysis. Liver was homogenized in 24 volumes of tris-EDTA buffer and the cytosol was obtained by ultracentrifugation. A portion of the supernatant (1 ml) was mixed with eight concentrations of $[^3H]E_2$ with and without $10^{-6}M$ DES in dialysis bags. Each of the 16 dialysis bags was suspended in 50 ml of the buffer containing the corresponding concentrations of radioactive estradiol with and without DES. After magnetic stirring for 24 hours in the cold room (4°C), binding (in quadruplicate 0.2-ml samples of the dialyzed cytosol) was measured by gel filtration. The binding is depicted in the absence of DES (open circles), in the presence of DES (squares), and as the difference (solid circles). The brackets indicate the SEM. The inset is a Lineweaver-Burk plot of the difference. The reciprocal of the estradiol concentration is the abscissa, and the reciprocal of the estradiol bound is the ordinate. The line is a least squares fit of the data.

the supernatant and in an extract (with a mixture of $2M$ NaCl and $5M$ urea) of highly purified nuclei (Table 2A). Temperature-dependent macromolecular binding in the nucleus is present. Addition of an excess of nonradioactive estradiol or DES diminishes both supernatant and nuclear macromolecular binding. In other experiments liver supernatant had previously been incubated with $[^3H]E_2$ and was then incubated with crude liver chromatin preparations (Table 2B). The chromatin was sedimented and washed, and the macromolecular binding was measured by gel filtration of a salt-urea extract. Macromolecular binding to chromatin is dependent on supernatant binding in a temperature-related process.

This hepatic estrogen-binding macromolecule is not species specific to rat. We have found macromolecules with high affinity for binding estrogen in the liver supernatant of the mouse, rabbit, and green monkey. The binding of estradiol to monkey liver can be distinguished from its interaction with potentially contaminating sex hormone–binding globulin from serum by the addition of competitors. Sex hormone–binding globulin binds estradiol and testosterone, but not DES, with high affinity (*7*). The binding in monkey liver cytosol (13,500 dpm in 0.2 ml or 5,200 dpm per milligram of cytosol protein) is not diminished in the presence of $10^{-7}M$ testosterone but is diminished to 15 percent of control in the presence of $10^{-7}M$ DES.

Previous studies have not shown specific binding of estrogen in the supernatant fraction of the liver of a mammal after the neonatal period (*2*). There are several factors that contribute to the inability of previous studies to demonstrate specific binding of estradiol in liver supernatant. Binding of estradiol by the liver is relatively low compared to that in the uterus and is predominately in the 4*S* instead of 8*S* region as is found with uterine supernatant. The concentration of estradiol in liver supernatant is diminished by metabolism, especially in prolonged incubations or at elevated temperatures. The most important factor in previous studies of the rat was that the animals used were prepubescent.

The binding of estradiol in liver supernatant is much lower in the prepubescent female rat than in the adult (see Fig. 2). In the same experiment we studied the effect of the administration of estrogen on plasma renin substrate. (Estrogen administration is known to increase plasma renin substrate in adult rats.) As shown in Fig. 2, administration of ethinyl estradiol increases the concentration of the plasma protein renin substrate severalfold in the adult rat, but has little effect on this substrate in the prepubescent rat. Plasma renin substrate was measured by radioimmunoassay of angiotensin I generated in the presence of an excess of purified rat kidney renin (*8*). The level of plasma renin substrate is also regulated by glucocorticoids (*8*). We observed that administration of the potent glucocorticoid dexamethasone increases plasma renin substrate to the same extent in the prepubescent rat as in the adult. This indicates that renin substrate responsiveness to administration of estrogen is preferentially low in the prepubescent. Compared to the adult, the prepubescent male also has much lower specific binding of estrogen in liver supernatant and is unresponsive with changes in plasma renin substrate after administration of estrogen but not of glucocorticoid. Thus, the development of the estrogen-binding protein in the liver of the rat correlates with the ability of estrogen administration to increase plasma renin substrate.

The estrogen induction of increased levels of plasma renin substrate is most likely due to a direct effect on the liver, increasing hepatic synthesis of the plasma protein. Administration of an estrogen in vivo or directly in the perfusate increases the synthesis of renin substrate in the isolated perfused rat liver (*9*).

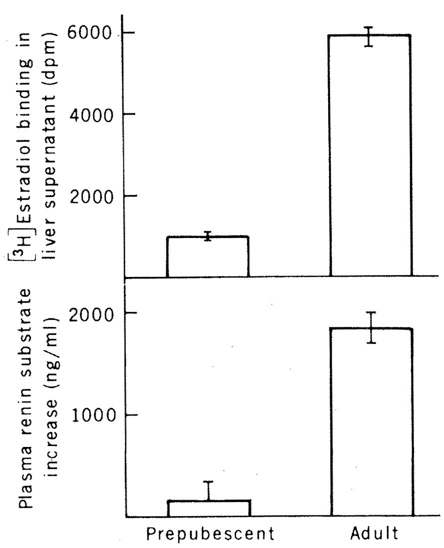


Fig. 2. Developmental correlation of estrogen binding in liver and estrogen induction of plasma renin substrate. Macromolecular binding of $2 \times 10^{-9}M$ $[^3H]E_2$ was measured by gel filtration after incubation in ice for 1 hour in 0.2 ml of liver supernatant from 27-day-old prepubescent rats and 200-g adult female rats of the control groups (top). Groups of five animals each of prepubescent and adult rats received subcutaneous injections of 100 μg of 17α-ethinyl estradiol or the vehicle alone (propylene glycol as control) at 0 and 24 hours. At 48 hours plasma renin substrate was measured by radioimmunoassay. The control levels of plasma renin substrate were 1040 $\pm$ 80 ng/ml for the prepubescent group and 1100 $\pm$ 80 ng/ml for the adult group. The graph indicates the increase above control in the estrogen-treated groups (bottom). The bars represent the standard error of the mean.

In lower vertebrates administration of estrogen stimulates the synthesis of egg yolk proteins in the liver. These egg yolk proteins are then secreted into the plasma and deposited in the follicles of the ovary (*10*). In some studies estrogen-binding proteins have been found in the supernatant of chicken and frog liver (*11*).

Administration of estrogen to women is known to increase the plasma concentration of several plasma proteins and decrease that of others (*3*). Women on estrogen-containing birth control pills are known to have increased clotting factors (*12, 13*), decreased clotting inhibitor antithrombin III (*12, 13*), increased renin substrate (*14*), and increased triglycerides and pre-β-lipoproteins (*15*). These proteins are synthesized in and secreted from the liver. Although their relative importance as initiating factors remains unresolved, increased clotting factors and decreased antithrombin III may contribute to thromboembolism, increased renin substrate may contribute to hypertension, and increased triglycerides and lipoproteins might accelerate atherosclerosis (*13, 16*).

The presence of the estrogen-binding protein in the liver of all four mammals studied suggests that the human liver might also contain this potential receptor. We need to pursue the possibility that these plasma protein changes and these potentially lethal side effects of estrogen-containing contraceptives might be avoided by modifications of the contraceptives designed to minimize interaction of the estrogen with its receptor in the liver.

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Human Newborns Differentiate Differing Concentrations of Sucrose and Glucose

Abstract. *One- to three-day-old infants who sucked to obtain flavored water revealed a precisely graded tongue movement which was sensitive to the gustatory properties of the flavored water. Stronger concentrations of sucrose and glucose elicited movements of greater amplitude than did weaker concentrations; sucrose was effective at lower concentrations than was glucose. These results correspond well with the relative sweetness adults attribute to these solutions in psychophysical studies.*

We have found that newborn human infants respond differentially to two concentrations of two sugars precisely as human adults do.

The traditional ascription of incompetence to the newly born (*1*) has met with some jolts in recent years. The newborn learns (*2*), he sees (*3*), he hears (*4*), and, after a while, he divides up the world of speech (*5*), color (*6*), and space (*7*) into the categories familiar to us all. We looked at the human newborn's responses to tasteful substances primarily because of our interest in the transformation of reflex into representation (*8*). Along the way, we found out that infants, in the first days after birth, are able to indicate with fair precision their differentiation among various sweet solutions.

We tested 75 healthy full-term infants with a variety of gustatory stimuli in a broad investigation of the effectiveness of the pressure-transducing nipple described below. The data reported here are from those ten infants who received at least one other sugar concentration besides the standard glucose given to all infants. All infants tested were between 1 and 3 days of age.

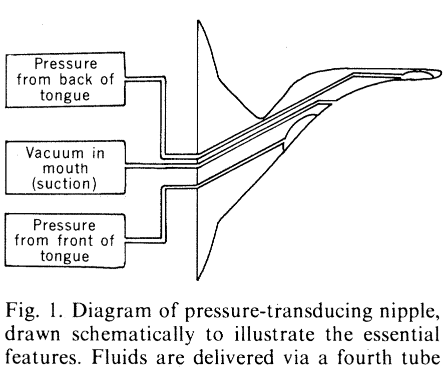

Fig. 1. Diagram of pressure-transducing nipple, drawn schematically to illustrate the essential features. Fluids are delivered via a fourth tube parallel to (behind, in this drawing) that shown for measuring vacuum in the mouth.

The recording nipple depicted schematically in Fig. 1 was constructed of medical grade Silastic rubber, and was designed to record independently the pressures exerted by the front and back of the infant's tongue, as well as the negative pressure created in the infant's mouth during sucking. The rationale for these several dependent variables is described elsewhere (*8*) in a theoretical discussion of the relative contribution to palatability of ingestive and rejective reflexes from the front and back portions of the mammalian tongue. Each channel of the recording nipple was connected to a Statham P23A pressure transducer, the output of which was amplified and recorded with a Grass model 7 polygraph. Sterile solutions were delivered to the infant's mouth through a fourth tube in the nipple parallel to that illustrated for measuring the vacuum in the infant's mouth. This tube connected the oral cavity directly to the fluid reservoir, which was kept at the level of the infant's mouth, so that negative pressure in the mouth directly delivered fluid to the mouth.

All infants were from a nursery in Yale–New Haven Hospital in which feedings were not scheduled by the clock; most were tested 3 to 5 hours after their previous feeding. The experimenter entered the nursery, where a nurse checked the infant for soiled diapers; the experimenter then carried the re-diapered infant to the experimental room in the newborn special care unit. The seated experimenter cradled the infant in his left arm and offered the nipple with his right hand. A second experimenter managed the polygraph and filled the sterile glass reservoir with 2 ml of the solution to be tasted. A "trial" lasted until the 2 ml were consumed or until the infant did not suck at all for 2 minutes. All infants offered sugar solutions under the paradigm described here rapidly consumed the 2 ml offered of each concentration. Five percent (0.277*M*) glucose was offered first to all babies, to provide a standard of response amplitude to which other solutions could be compared. The three other sugar concentrations we employed were 10 percent (0.555*M*) glucose, 2 percent (0.058*M*) sucrose, and 4 percent (0.117*M*) sucrose. Infants generally received three solutions other than the standard, but in some cases, reported elsewhere (*9*), these included mild solutions of sodium chloride or quinine hydrochloride, rather than all of the sugar solutions (*10*). Other infants, observed for their reactions to different versions of the recording nipple, received only the standard.

The results of major interest are summarized in Fig. 2. The anterior tongue pressure score is derived by taking the mean amplitude of the strongest ten sucks to a given concentration, and dividing that by the mean amplitude of the strongest ten sucks to the standard glucose concentration for that infant. Standard errors are in-

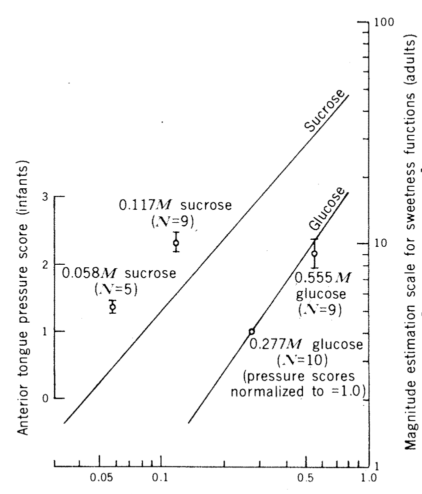

Fig. 2. The four data points are tongue pressure scores (left ordinate) for the four sugar solutions given to infants in this study. Standard errors are indicated by vertical bars. The two lines are psychophysical functions (right ordinate) for adults, from Moskowitz (*11*).