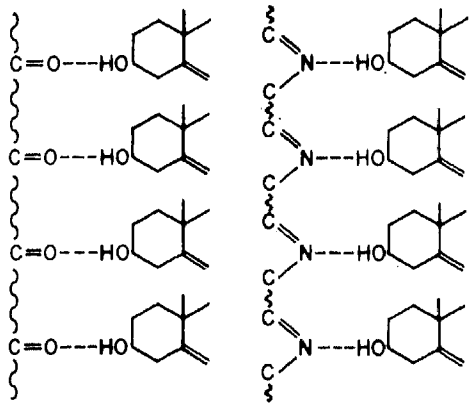
oils at saturation in two states of dispersion, a less stable form that yields insoluble clathrates with appropriate dicarboxylic acid or imidazole and a more stable form that does not yield clathrates. The results would suggest that at saturation the two forms are present in equal amounts. Obviously some physico-chemical determination must be employed to substantiate or refute this explanation with certainty.

It would appear from the specificity studies that have been carried out(1,2) that the clathrate-forming agent must be of such a molecular size that the susceptible cholesterol molecules are held at a favorable spacing for crystallization, presumably involving hydrogen bonding, to occur. This concept is shown diagrammatically as follows:



Although it is unlikely *in vivo* that tissue concentrations of free dicarboxylic acids or imidazole would ever exist that would be involved in clathrate formation, it is conceivable that hydrogen bond formation between cholesterol and tissue components containing regularly spaced atoms capable of sharing the hydroxyl hydrogens of cholesterol could occur. If such a phenomenon is the explanation for the precipitation of cholesterol under certain pathological conditions, it would appear that insoluble clathrate formation would be restricted to free cholesterol occurring in tissue lipids over and above one-half of saturation. Practical methods for the prevention of undesirable cholesterol deposits might be directed to means of keeping the cholesterol concentration of body fats below the level of one-half of saturation.

Summary. Insoluble-clathrate formation occurs between cholesterol and certain dicarboxylic acids or imidazole in a number of natural or synthetic triglycerides only when the initial concentration of cholesterol is in excess of one-half of saturation. The possibility that cholesterol is present in oils in two separate states of dispersion is discussed.

1. Wright, L. D., Presberg, J. A., Fed. Proc., 1963, v22, 269.

2. ———, Proc. Soc. Exp. Biol. and Med., 1964, v115, 497.

Received September 9, 1965. P.S.E.B.M., 1966, v121.

A Deficient Binding Mechanism for Norepinephrine in Hearts of Scorbutic Guinea Pigs. (30754)

NGEM B. THOA,* RICHARD J. WURTMAN AND JULIUS AXELROD
(Introduced by Sidney S. Chernick)
Laboratory of Clinical Science, National Institute of Mental Health, National Institutes of Health, Bethesda, Md.

It has been demonstrated that the induction of scurvy in guinea pigs is associated with hyperresponsiveness to the pressor and cardiac inotropic effects of injected catecholamines(1).

Such hyperresponsiveness could be the consequence of at least 3 different mechanisms: *1*) A larger fraction of the circulating exogenous catecholamine could be delivered to the cardiovascular receptors(2), due to the vascular changes produced in scurvy. *2*) The

* Visiting Scientist. Present address: Dept. of Pharmacology, Howard Univ. Med. School, Washington, D.C.

efficiency of the processes by which catecholamines are inactivated in the locale of the receptor could be impaired. This inactivation normally proceeds by two mechanisms, enzymatic O-methylation(3), or uptake and binding of the chemically unchanged catecholamine within sympathetic nerve endings(4,5). 3) The sensitivity of the receptor could be altered.

It will be shown that the dietary induction of scurvy is associated with a decrease in the efficiency with which the guinea pig heart takes up or retains circulating H^3-norepinephrine.

Materials and methods. Adult male albino guinea pigs initially weighing 350 to 400 g were maintained on a scorbutogenic diet (Nutritional Biochemicals Corp.) for 3 to 4 weeks; control animals received ascorbic acid supplements in addition to this diet. At the end of this period, the scorbutic animals had developed tender joints, which showed changes typical of scurvy on histologic examination (performed by Dr. Leon Sokoloff, Nat. Inst. of Arthritis and Metabolic Diseases, Bethesda, Md.). The scorbutic animals were shown to be hyperresponsive to injected norepinephrine by procedures previously described(1), *i.e.*, the increments in cardiac contractile force and in blood pressure following norepinephrine injection were enhanced.

Groups of 9 guinea pigs received 40 μc/kg of H^3-norepinephrine (7.2 c/mmole, New England Nuclear Corp., 40 μc/ml) into the jugular vein, and were killed 40 minutes later. The hearts were immediately removed and assayed for H^3-norepinephrine by a procedure described previously(4). In another group of guinea pigs, the endogenous cardiac norepinephrine was assayed by the method of Anton and Sayre(6).

The fraction of the cardiac output delivered (*via* the coronary arteries) to the hearts of scorbutic and normal animals was estimated by the method of Sapirstein(7). Groups of guinea pigs were given 1 μc of $K^{42}Cl$ intravenously (4 meq/l, Iso-Serv Corp., Cambridge, Mass.) in 0.5 ml of isotonic saline, and killed 15 seconds later. The hearts were weighed and their K^{42} content was measured in a gamma well scintillation counter, as described before(2).

Catechol-O-methyl transferase was assayed using S-adenosylmethionine-C^{14} by procedures previously described(8), and monoamine oxidase was estimated by the method of Wurtman and Axelrod(9).

Results and discussion. Injected H^3-norepinephrine disappears rapidly from the circulation; hence most of a given dose that is eventually to act on the heart reaches the myocardium in its first few transits through the circulation(2). It has been shown that, when small doses of norepinephrine are administered to an animal, the percent of the dose delivered to the myocardium is a linear function of the fraction of the cardiac output which perfuses the coronary arteries(2). It was possible that scurvy enhanced the responses of the heart to a given dose of catecholamine by increasing the cardiac fractional perfusion, thereby increasing the fraction of injected norepinephrine which actually reached the heart. Thus cardiac fractional perfusion was estimated in normal and scorbutic animals by the method of Sapirstein (7). It was found (Table I) that scurvy produced no alteration in the percent of the arterial blood which was delivered to the heart, indicating that scorbutic and normal hearts receive essentially the same fractions of the tracer dose of catecholamine.

TABLE I. Delivery and Retention of Circulating H^3-Norepinephrine (H^3-NE) in Hearts of Normal and Scorbutic Guinea Pigs.

Groups of 9 guinea pigs were given 40 μc/kg of H^3-norepinephrine intravenously and killed 40 min later. To estimate cardiac fractional perfusion, other animals were given 1 μc of $K^{42}Cl$ intravenously, and killed 15 sec later. Data are expressed as mean ± standard error.

	1	2	3
Treatment	% cardiac output delivered to heart	% injected H^3NE bound per heart	% delivered H^3NE retained by heart
Control	1.65 ± .03	.443 ± .026	27.0 ± 1.3
Scorbutic	1.68 ± .02	.371 ± .022*	22.0 ± .9*

* $P < 0.01$.

Column 1: % of injected $K^{42}Cl$ present in hearts after 15 sec.

Column 2: % of injected H^3NE present in hearts after 40 min.

Column 3: Column 2 divided by Column 1 (× 100).

The capacity of guinea pig hearts to inactivate circulating H^3-norepinephrine by uptake and binding within sympathetic nerve endings was estimated by measuring cardiac H^3-norepinephrine contents 40 minutes after injection of the neurohumor. It has been shown that at this time after injection, almost all of the H^3-norepinephrine has left the circulation and is bound within sympathetic nerve endings in organs such as heart, in a physiologically-inert form. Since scorbutic and normal hearts both received the same fraction of the injected norepinephrine (*e.g.*, 1.65-1.68%), it could be expected that they would also take up and bind a similar proportion of the neurohumor, unless scurvy interfered with the uptake or binding mechanisms. It was observed that normal hearts contained 0.443% of the injected H^3-norepinephrine (27% of the amount initially delivered), while scorbutic hearts contained only 0.371% of the injected neurohumor (22% of the amount delivered). The smaller percentage of delivered H^3-norepinephrine present in the scorbutic heart would suggest a deficiency in the processes responsible for the uptake or binding of the catecholamine.

The final weights of normal guinea pigs were considerably greater than those of the scorbutic animals (540 *vs* 260 g); thus they were given a correspondingly larger dose of H^3-norepinephrine, and about twice as much actual catecholamine was delivered to their hearts. It seems unlikely that this difference in dosage was responsible for the binding deficit noted here, since it has been demonstrated repeatedly that normal hearts bind and retain a relatively constant fraction of a dose of injected H^3-norepinephrine throughout a dosage range considerably wider than the 2-fold range used here(10).

These observations indicate that the supersensitivity to catecholamines seen in scorbutic guinea pigs is correlated with decreased inactivation of the circulating amine by binding. Consequently there is probably a larger proportion of free, physiologically-active catecholamine present at the cardiac receptor sites.

The endogenous cardiac catecholamine content was also examined in the scorbutic guinea pig. There was a significant decrease in the norepinephrine level of the ascorbic acid-deficient heart, whether the results were expressed in terms of total heart or as concentration (Table II). The lower concentration of the endogenous catecholamine also may reflect the reduced ability of the scorbutic heart to bind norepinephrine (after it is synthesized locally or is taken up from the circulation).

TABLE II. Endogenous Norepinephrine in Hearts of Normal and Scorbutic Guinea Pigs.

	Heart wt/g	Norepinephrine μg per heart	Norepinephrine μg per/g heart
Normal	1.43 ± .12	2.09 ± .19	1.46 ± .16
Scorbutic	.96 ± .05†	.90 ± .17†	.94 ± .15*

* $P<.05$. † $P<.001$.
Results are expressed as the mean ± SEM of groups of 4 normal and 4 scorbutic guinea pigs.

Cardiac catechol-O-methyl transferase and monoamine oxidase activities were not altered by scurvy.

These observations suggest that the cardiac supersensitivity to catecholamines found in scurvy shares a common mechanism (reduced ability to inactivate catecholamines by binding) with that produced by hyperthyroidism (2), cocaine administration(11), or sympathetic denervation(5).

Summary. There is a reduced ability to take up and bind H^3-norepinephrine in the hearts of scorbutic guinea pigs. There is also a reduction in the level of endogenous norepinephrine in these hearts. These observations indicate that the supersensitivity to catecholamines found in scurvy may be due in part to the reduced ability to inactivate catecholamines by uptake and binding.

1. Thoa, N. B., Booker, W. M., Fed. Proc., 1963, v22, 448.
2. Wurtman, R. J., Kopin, I. J., Axelrod, J., Endocrinology, 1963, v73, 63.
3. Axelrod, J., Tomchick, R., J. Biol. Chem., 1958, v233, 702.
4. Whitby, L. G., Axelrod, J., Weil-Marlherbe, H., J. Pharmacol., 1962, v132, 193.
5. Hertting, G., Axelrod, J., Kopin, I. J., Whitby, L. G., Nature, 1961, v189, 66.
6. Anton, A. H., Sayre, D. F., J. Pharmacol., 1962, v138, 360.

7. Sapirstein, L. A., Am. J. Physiol., 1958, v193, 161.
8. Axelrod, J., Albers, W., Clemente, C. D., J. Neurochem., 1959, v5, 68.
9. Wurtman, R. J., Axelrod, J., Biochem. Pharmacol., 1963, v12, 1439.
10. Gillis, C. N., ibid., 1964, v13, 1.
11. Whitby, L. G., Hertting, G., Axelrod, J., Nature, 1960, v187, 604.

Received September 11, 1965. P.S.E.B.M., 1966, v121.

Concentrations of Uric Acid in the Sweat of Control and Mongoloid Children. (30755)

RONALD A. DANTON AND WILLIAM L. NYHAN
(Introduced by William J. Harrington)
Department of Pediatrics, University of Miami School of Medicine, Miami, Fla.

The presence of uric acid in human sweat was reported some time ago on the basis of studies employing colorimetric methods(1,2, 3). However, it has more recently been reported that uric acid could not be detected in sweat using the enzymatic spectrophotometric method(4). In the course of studies on the metabolism of uric acid in a syndrome of uric acid overproduction and central nervous system dysfunction(5), uric acid was found in thermal sweat from a patient with this syndrome. It appeared of interest, therefore, to investigate the presence or absence of this compound in the sweat of children who did not have this disorder.

It was found that appreciable quantities of uric acid could be detected in sweat using the enzymatic spectrophotometric method.

Materials and methods. The subjects ranged in age from 8 months to 21 years. They were: convalescent pediatric patients in the Jackson Memorial Hospital; ambulatory retarded children from the Haven School; and a volunteer student laboratory assistant. The subjects were all healthy at the time of the experiment and were eating regular diets. The population of retarded children at the Haven School contains a relatively large proportion of mongoloid children, and it became evident early in this study that they should be considered as a separate group. Nonmongoloid retarded children from the same institution were included among the controls.

Sweat was collected in plastic bags after the subjects were given a bath with soap, rinsed with tap water, then with distilled water, and dried. Two types of bags are in use in the laboratory: a full body bag in which the subject's entire body except the neck and head are contained, and a half body bag in which the area from the lower neck to waist is contained. The bags were made in a variety of sizes. Once in the bag, the subject was covered from neck to toe with blankets. The experiment was usually performed in rooms without air-conditioning. It was possible to collect as much as 700 ml in 1 hour with full body bag and 100 ml with the half body bag. The sweat was collected from the inside of the bag with a syringe, centrifuged, decanted, and frozen until tested.

Concentrations of uric acid were assayed using the enzymatic spectrophotometric method(6). The amounts of sweat used in the cuvette ranged from 0.25 to 1.0 ml, depending on the optical density.

Results. Appreciable quantities of uric acid were detected in the sweat of each of 29 subjects studied (Table I). The lowest concentration obtained in this series was 0.09 mg/100 ml.

These data represent collections of sweat

TABLE I. Uric Acid Concentration—Sweat.

	No. of subjects	Uric acid (mg/100 ml)
Mongoloid	13	.637 ± .156
Control	16	.202 ± .029
	t = 3.02	P < .01

The values are mean concentrations ± standard errors.