Table 1. Separation by centrifugation in 2-bromoethanol. For description of the zones see text. The aliquot for zone B was taken from the bottom of the zone. In each case 400 squares were counted and the particles per cubic centimeter were calculated from the counts.

Depth (mm)	Zone	Organic particles	Inorganic particles (10^9/cm^3)
10	A	Few	None noted
40	B	Too dense to count	None noted
45	C	None noted	0.015
50	C	None noted	.023
55	C	None noted	.007
60	C	None noted	.0
65	C	None noted	.038
70	C	None noted	.045
75	C	None noted	.030
80	D	None noted	Too dense to count

ume) showed a reduction of cells from 300×10^6 per milliliter to 291×10^6 per milliliter, a change well within the sampling error.

Visual examination of a centrifuge tube after density-gradient centrifugation showed four distinct zones. Typical results of microscopic examination of the aliquots from various levels in the gradient are listed in Table 1. Virtually no particulate material remained in the clear upper zone (A), which corresponded to the aqueous part of the original particulate suspension. Below, zone B was a dense concentration of organic particles in the gradient formed between the water and the 2-bromoethanol. Below this particulate zone was zone C, nearly devoid of particulate material, which corresponded to the 50 ml of 2-bromoethanol. The bottom band, zone D, below the 2-bromoethanol, was a pellet of concentrated particulate inorganic material. At the lower limits of resolution, differentiation of organic from inorganic particles becomes difficult.

In order to determine further whether particulate organic material could penetrate into the 2-bromoethanol zone of a density gradient, tests were conducted with pure cultures of *E. coli*. The experiments were conducted exactly like the ones described above except that no inorganic material was included in the aqueous particulate suspension. No bacterial cells were present in the 2-bromoethanol zone after centrifugation.

Table 2. Separation by sucrose density-gradient centrifugation. There were 1116 billion organic and 192 billion inorganic particles per cubic centimeter in the uncentrifuged particulate suspension. The 74-percent sucrose zone was used as the separation point of organic from inorganic material. This was centrifuged at 1040*g* for 60 minutes. Each value in columns 2 and 3 was calculated from a count of 80 squares.

Sucrose (%)	Organic particles (10^9/cm^3)	Inorganic particles (10^9/cm^3)
60	7.47	0.525
70	4.08	2.505
74	3.15	3.150
80	1.83	4.005

The best results obtained with a sucrose density gradient are presented in Table 2. In this case 99.7 percent of the organic particles remained in the density zone above the 74-percent sucrose level and 98.4 percent of the inorganic particles were in a zone below the 74-percent sucrose (*1*). The separation obtained with 2-bromoethanol is significantly better, since no organic particles were counted in the 2-bromoethanol at 5 mm below the bottom of the organic pellet, and two orders of magnitude fewer inorganic particles remained suspended in the 2-bromoethanol than were found above the 60-percent sucrose level (*1*).

The isolated organic fraction was removed from the gradient tube by aspiration, after which the remaining material was removed and saved; this was the inorganic fraction of the original sample. The particulate material from both fractions was then isolated from the liquid phase by recentrifugation and aspiration (*1*).

Thus, the use of 2-bromoethanol for density-gradient separation results in very sharp separations of isolated fractions. This system has the advantage of a density so high that no organic particle can pass through it unless associated with an inorganic particle. Such association is precluded as far as possible by homogenization before centrifugation. Further advantages are the low interfacial forces in the water-bromoethanol gradient zone and the relatively low viscosity of the 2-bromoethanol so that no inorganic particles are held back by either interfacial forces or viscosity. Neither an equilibration period nor a gradient engine is required.

This work has concerned particles of microscopic dimensions (0.5 to 200.0 μ); it is obvious that much higher fields would be required for colloidal particles (*9*).

W. T. LAMMERS

Davidson College,
Davidson, North Carolina

References and Notes

1. W. T. Lammers, *Limnol. Oceanog.* **7**, 224 (1962).
2. ———, in "Status report No. 3 on Clinch River study," Oak Ridge Natl. Lab., Rept. ORNL-3370, R. J. Morton, Ed. (1962), p. 78.
3. ———, paper presented at the 15th International Congress of Limnology, Madison, Wis.
4. J. Lederberg, *Science* **132**, 393 (1960).
5. A. F. Reid and A. H. Halff, *Science* **135**, 319 (1962).
6. P. L. Kirk, *Crime Investigation, Physical Evidence, and the Police Laboratory* (Interscience, New York, 1953), p. 285.
7. N. G. Anderson, in *Physical Techniques in Biological Research, III*, G. Oster and A. W. Pollister, Eds. (Academic Press, New York, 1959), p. 299; R. Ballentine and D. D. Burford, *Anal. Biochem.* **1**, 263 (1960); C. de Duve, J. Berthet, H. Beaufay, in *Progress in Biophysics and Biophysical Chemistry*, J. A. V. Butler and B. Katz, Eds. (Pergamon Press, New York, 1959), vol. 9, p. 325.
8. American Public Health Assn., *Standard Methods for the Examination of Water and Waste Water* (A.P.H.A., New York, ed. 11, 1960), p. 457.
9. I thank Dr. N. G. Anderson, Biology Division, ORNL, for suggesting a trial of 2-bromoethanol and for his advice and guidance. This work was done as a part of subcontract 2143 from Oak Ridge National Laboratory operated by the Union Carbide Nuclear Corporation for the U.S. Atomic Energy Commission.

5 February 1963

Norepinephrine Synthesis from Tyrosine-C^{14} in Isolated Perfused Guinea Pig Heart

Abstract. *The isolated, perfused guinea pig heart contains all the catalysts required to form norepinephrine from the dietary precursor, tyrosine. The conversion of tyrosine-C^{14} to norepinephrine in the perfused heart occurred at a rate comparable to that estimated for this conversion in vivo. To account for the maintenance of norepinephrine stores in the normal heart, it is not necessary to postulate that the hormone is extracted from the blood.*

Norepinephrine is a normal constituent of the heart, presumably associated with its sympathetic innervation. Although certain extra-adrenal tissues and sympathetic nerves can perform some of the biochemical conversions required for the synthesis of norepinephrine (*1*), the question has never been answered as to the origin of this hormone in the heart. Recent studies on the uptake by the heart of administered radioactive norepinephrine (*2*) give the impression that endogenous stores of cardiac norepinephrine are normally maintained by extraction from the blood. It is conceivable that under certain conditions uptake from the blood may be of some significance. However, the studies reported here show that the heart itself is capable of synthesizing norepinephrine

Table 1. Radioactivity in tyrosine, norepinephrine, and dopamine in the perfused guinea pig heart. Specific activities are in counts per minute (cpm) per millimicromole.

(*T*) Perfusion time (hrs)	Tyrosine in perfusate		Norepinephrine in heart			Dopamine in heart (cpm/g of tissue)	Free tyrosine in heart (specific activity)
	(*A*) Specific activity	μg/ml*	Specific activity	(*B*) (cpm/g of tissue)	(*C*) Rate of formation†		
1.5	16,700	0.33	1090	7,480	0.05	1050	701
2	31,761	.15	1800	12,800	.03	1840	2040
2	14,220	.23	1130	5,270	.03	850	1730

*The carrier free tyrosine in the perfusate (0.03 μg/ml) was diluted with tyrosine released from the heart. The amount of tyrosine released varied from experiment to experiment but in any one experiment the specific activity of tyrosine in the perfusate attained constant values early in the perfusion period. † In micrograms per gram of tissue per hour, calculated from the equation $C = (B/A) \times 0.169 \times (1/T)$. (Molecular weight of norepinephrine is 169.)

from the dietary precursor, tyrosine, and at a rate which is consistent with estimated rates of formation in vivo.

Guinea pig hearts were perfused by the Langendorff technique with oxygenated Tyrode's solution at 37°C, *p*H 7.4 and a pressure of 55 cm of water. Perfusion flow rates were about 8 to 10 ml/min. After an equilibration period of 10 minutes, 200 ml of Tyrode's solution containing 10 μc of tyrosine-C^{14} (300 μc/μmole, uniformly labeled; 0.03 μg/ml) was introduced as the perfusion fluid, and the hearts were continuously reperfused for 1½ to 2 hours.

After perfusion, each heart (0.75 to 0.90 g) was homogenized in 10 ml of 5 percent trichloroacetic acid and centrifuged. The supernatant solutions were treated with alumina to adsorb catecholamines; tyrosine in the effluents from the alumina columns was in turn adsorbed on Dowex-50 (H^+); it was then eluted with a solution of 3*N* NH_3. After removal of the ammonia, the tyrosine-containing solutions were passed over an IRC-50 column (buffered with ammonium acetate, *p*H 5) to remove basic metabolites such as tyramine and normetanephrine. Aliquots of the perfusates and of the effluents from the IRC-50 columns were taken for tyrosine assay (*3*) and for radioassay (*4*). The alumina columns were then eluted with 0.2*N* acetic acid, and an aliquot of the eluate was assayed for norepinephrine by a modification of the trihydroxyindole method (*5*). To the remainder of each eluate was added 25 μg each of norepinephrine and 3,4-dihydroxyphenethylamine (dopamine), as carriers. This mixture was placed on a Dowex-50 (H^+) column and eluted first with 1*N* HCl to obtain norepinephrine and then with 2*N* HCl for dopamine (*6*).

Small aliquots were used for determining norepinephrine and dopamine by fluorometric assay; to determine the radioactivity, the remainder was dissolved in Bray's solution (*4*) for scintillation counting. Further identification of the norepinephrine formed during perfusion was obtained by paper chromatography (Fig. 1). The radioactivity migrated at the same speed as authentic norepinephrine. Furthermore, all the material eluted by 1*N* HCl from the Dowex-50 columns appeared almost quantitatively in the norepinephrine area of the chromatograms.

Three experiments are summarized in Table 1. Perfusion with tyrosine resulted in the formation of norepinephrine and dopamine. However, the dopamine (representing about 15 percent of the total catecholamines) has not yet been identified with as much certainty as the norepinephrine.

The data in Table 1 also show calculated values for the formation of norepinephrine in the three experiments. These values (0.03 to 0.05 μg/g per hr) were based on the specific activity of the tyrosine in the perfusate. Calculations could also have been based on the free tyrosine in the heart (last column). However, equilibration was not attained between the perfusate tyrosine and free tyrosine in the heart during the period of perfusion. Had the latter values been used, estimated values for the formation of norepinephrine would have been 10 to 20 times greater. Such high rates of formation would have resulted in a net increase in total heart norepinephrine which was not observed. One must assume, then, that the tyro-

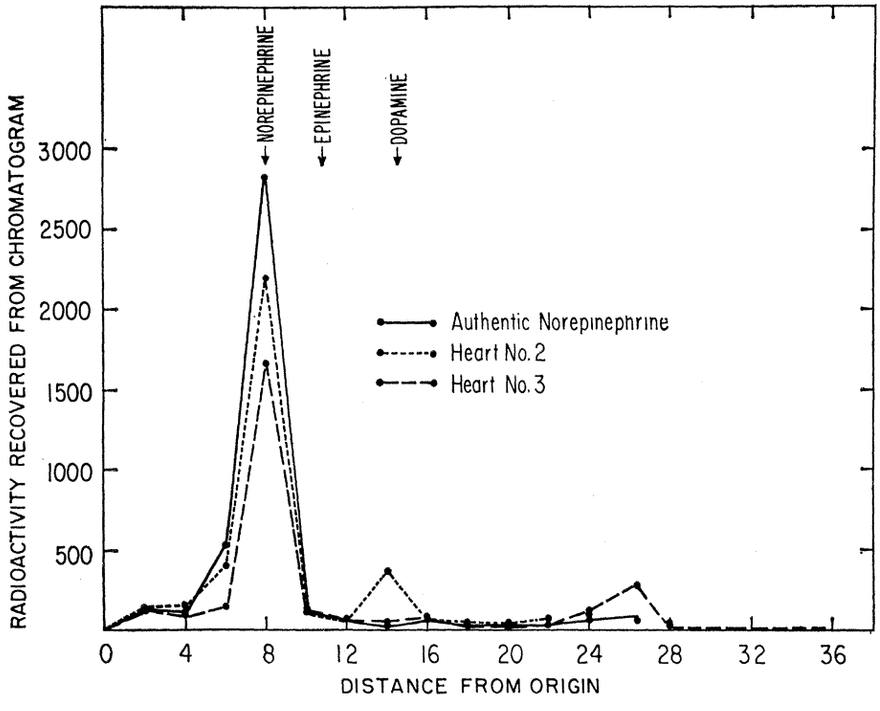

Fig. 1. Paper chromatograms of apparent norepinephrine isolated from perfused guinea pig heart. The radioactivity recovered (ordinate) is counts per minute; distance from origin (abscissa) is centimeters; heart No. 2, 2400 count/min applied; No. 3, 1900 count/min applied. Norepinephrine was extracted from the Bray's solution, purified by Dowex-50 (H^+) chromatography, then chromatographed on Whatman No. 1 paper; n-butanol: 1*N* HCl (1:1; organic phase). Catecholamine areas were detected by the pink color of the aminochromes formed in an NH_3 atmosphere and each chromatogram was cut into 2 cm sections and placed into a counting vial containing 1 ml of 0.01*N* HCl; 15 min later 10 ml of Bray's solution was added.

sine in the perfusate was the source of the norepinephrine with but little dilution by tyrosine in the bulk of heart. Of course, only a fraction of the heart tissue represents sympathetic nervous tissue.

The estimated rates of formation, shown in Table 1, are probably too low for two reasons. First, norepinephrine was continuously released from the heart and appreciable amounts of acidic metabolites of norepinephrine were detected in the perfusate. Second, the tyrosine concentrations in the perfusate ranged from 0.15 to 0.33 μg/ml as compared to a normal plasma level of about 10 to 15 μg/ml. It is quite possible that the rate could have increased with larger concentrations of precursor. Estimates on the rate of synthesis of norepinephrine in intact mammalian heart have ranged from 0.03 to 0.2 μg/g per hour (*7*). It is apparent therefore that the rate of synthesis by the isolated perfused guinea pig heart is at least comparable to that reported in the intact animal.

Thus, the isolated heart contains all the catalysts required for converting tyrosine to norepinephrine:

tyrosine ⟶ dopa ⟶
dopamine ⟶ norepinephrine

Furthermore, norepinephrine synthesis could take place in each sympathetically innervated organ including heart, spleen, brain and blood vessels. These results reaffirm the widely held concept that norepinephrine, in contrast to epinephrine, is a local hormone.

SIDNEY SPECTOR, ALBERT SJOERDSMA
PEROLA ZALTZMAN-NIRENBERG
MORTON LEVITT
SIDNEY UDENFRIEND
National Heart Institute,
National Institutes of Health,
Bethesda, Maryland

References

1. McC. Goodall and N. Kirschner, *Circulation* **17**, 366 (1958); S. Udenfriend and C. R. Creveling, *J. Neurochem.* **4**, 350 (1959).
2. J. H. Burn and M. J. Rand, *J. Physiol.* **150**, 295 (1960); L. G. Whitby, J. Axelrod, H. Weil-Malherbe, *J. Pharmacol. Exptl. Therap.* **132**, 193 (1961); J. R. Crout, *Pharmacologist* **4**, 168 (1962).
3. T. P. Waalkes and S. Udenfriend, *J. Lab. Clin. Med.* **50**, 733 (1957).
4. A. G. Bray, *Anal. Biochem.* **1**, 279 (1960).
5. J. R. Crout, *Std. Methods Clin. Chem.* **3**, 62 (1961).
6. A. Bertler, A. Carlsson, E. Rosengren, *Acta Physiol. Scan.* **44**, 273 (1958).
7. R. Montanari, E. Costa, B. B. Brodie, *Pharmacologist* **4**, 179 (1962); S. Spector, K. Melmon, A. Sjoerdsma, *Proc. Soc. Exptl. Biol. Med.* **111**, 79 (1962); S. Udenfriend, unpublished observations.

9 January 1963

Indium Antimonide: the Metallic Form at Atmospheric Pressure

Abstract. *The crystal structure of metallic indium antimonide at atmospheric pressure and −197°C is essentially identical with that of white tin at 26°C.*

Compression of group IV elements and of group III–V and group II–IV binary compounds transforms them into metals (*1–3*). The phase change is marked by a large increase in density and a rise in the number of equivalent near neighbors from four, which is characteristic of these materials at low pressure, to six, which is characteristic of the new phase (*4*). The work of Drickamer and his co-workers (*3*) and of Kennedy and his co-workers (*1*) has clearly demonstrated the generality of the phenomenon.

Jamieson has examined the crystal structure of the metallic form of indium antimonide under high pressure and found it to be analogous to that of white tin. We now report a low-pressure study that shows the structure to be essentially identical with that of ordinary metallic tin.

The metallic form of InSb may be obtained at low pressures by cooling the material while it is under pressure and then reducing the pressure. The metallic form was first made as described previously (*1–3*) by application of pressures a few kilobars in excess of the transition pressure of 23 kb at a temperature of about 95°C. Periods of several hours were used to insure complete conversion.

Liquid nitrogen was then used to cool the entire assembly of press and sample. When the temperature of the sample had dropped to well below 210°K (−63°C) the pressure was released, and the sample was removed from the cylinder, which contained tungsten carbide. We found that the material was a very good metal with a very low resistance, comparable to that of aluminum at temperatures between 77° and 210°K. It was very shiny and metallic and extremely hard, somewhat like tool steel. It was found, empirically, to be stable for weeks, so long as it was kept at temperatures below −63°C, and it was even possible to machine it.

An x-ray diagram was taken by the Debye-Scherrer technique at 77°K. The spectrum with CuK α radiation is given in Table 1, together with the lattice spacings of ordinary white tin (*5*).

Table 1. Lattice spacings of white (or β tin) and metallic indium antimonide, InSb(II). The unit-cell dimensions for Sn(β) at 26°C and InSb (II) at −197°C are, respectively, *a*, 5.831 and 5.72 ± 0.16Å; *c*, 3.182 and 3.18 ± 0.03Å. The corresponding densities are, respectively, 7.286 and 7.54 ± 0.16 g/cm³.

hkl	Sn(β), d(Å), Cu. 1.5405 Å	InSb(II), d(Å), Cu. 1.5405 Å
200	2.915	2.90
101	2.793	2.78
220	2.062	2.05
211	2.017	2.02
301	1.659	1.65
112	1.484	1.48
400	1.458	
		1.44*
321	1.442	

* Unresolved.

It is clear from these data that the two structures are identical to within 0.02 Å in the spacings for the body-centered tetragonal lattice (*6*).

A. J. DARNELL
W. F. LIBBY
Department of Chemistry, University of California, Los Angeles 24

References and Notes

1. A. Jayaraman, R. C. Newton, G. C. Kennedy, *Nature* **191**, 1288 (1961); ———, "High pressure studies on diamond and zinc blende structures: melting and polymorphism," presented at the Diamond Symposium in Paris, May 1962.
2. B. J. Alder and R. H. Christian, *Phys. Rev. Letters* **8**, 367 (1961).
3. H. L. Suchan and H. G. Drickamer, *J. Chem. Phys.* **31**, 355 (1959); S. Minomura and H. G. Drickamer, *J. Phys. Chem. Solids* **23**, 451 (1962); G. A. Samara and H. G. Drickamer, *ibid.*, p. 457; ———, *J. Chem. Phys.* **37**, 408 (1962).
4. J. C. Jamieson, "Phase transitions under pressure of the semiconductor metal types and the earth's interior," Abstr. 1962 Ann. Meeting Geol. Soc. Am., p. 76A.
5. H. E. Swanson and E. Tatge, *Natl. Bur. Std. U.S. Circ.* **539** (1953), vol. 1, pp. 24–26.
6. Supported by the Directorate of Chemical Sciences, Air Force Office of Scientific Research under contract No. AF 49 (638)-901 and grant AF-AFOSR-245-63).

26 February 1963

Indium Antimonide: Superconductivity of the Metallic Form

Abstract. *The transition of metallic indium antimonide into the superconducting state begins at 2.1°K and is complete at about 1.6°K. These data are close to those for white tin.*

Superconductivity in metallic InSb, prepared and stabilized at atmospheric pressure in the way described by Darnell and Libby (*1*), has been observed.

Samples about 1 inch long and about