Table 1. Alkaloids recovered with a Soxhlet-type extractor and the new extractor. Each figure represents the average value of three assays (milligrams of alkaloid per 10 g sample of *D. stramonium*).

Maceration time (hr)	Alkaloid content of extract after 3 hr (mg)	
	Soxhlet	New extractor
½	27	30
1	29	30
2	30	33
24	38	35

together in an ultrasonic tank (*2*). Our new extractor was designed so that the sample could at the same time be subjected to ultrasonic energy and exposed to the recycling solvent; it has been used effectively for routine extractions of a variety of plant materials.

The extractor (Fig. 1) has four separable parts: a solvent or boiling flask *F*, connecting tube *E* with sintered-glass filter *C*, extraction flask *B*, and condenser *D*.

The sample to be extracted and the macerating solvent, if used, are put into the extraction flask *B* which may be placed either in an ultrasonic tank, if exposure to ultrasound is desired, or supported suitably for conventional extraction. For ultrasonic work the glass extraction flask was replaced with one made of hard polyethylene (*3*).

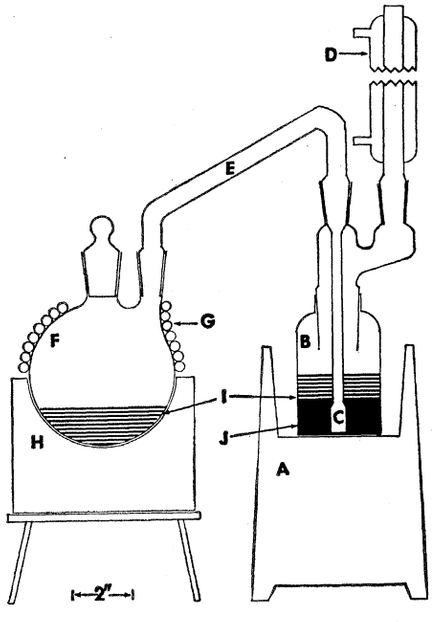

Fig. 1. Cross section of the new extractor: *A*, ultrasonic bath or suitable support; *B*, polyethylene or glass extraction flask; *C*, sintered-glass filter; *D*, condenser; *E*, glass connecting tube; *F*, solvent flask; *G*, cooling coils; *H*, heating mantle; *I*, solvent; *J*, material being extracted.

The extracting solvent is placed in flask *F*. The solvent flask is heated and cooled by connecting the heating mantle *H* to a two-cycle electric program timer. The duration and intensity of heating and cooling cycles must be experimentally determined for a particular solvent or mixture of solvents. During the heating cycle the solvent is vaporized and passes through the connecting tube and the sintered-glass filter before it enters the extraction flask. The solvent vapor condenses while flowing into the extraction flask and causes enough agitation of the ground plant material to permit considerable exposure to the solvent. When nearly all of the solvent has passed into the extraction flask the program timer initiates a cooling phase. During this phase, cold water, which is continually circulating through the coils *G* that surround the solvent flask, causes condensation of the remaining solvent vapor in the flask. The condensation results in the formation of a vacuum. The extract in flask *B* is siphoned through the connecting tube and into the solvent flask; the sintered-glass filter prevents passage of solid particles. When most of the extract has been removed from the extraction flask, air enters the tube at *C* and stops the flow of extract. The timer, if appropriately set, allows the process to be automatic and continuous.

The efficiency of the new extractor was compared with that of a Soxhlet extractor (Table 1). *Datura stramonium* samples were macerated in 80 ml of solvent (ether 20, alcohol U.S.P. 12, and ammonia 8 parts by volume) and extracted for 3 hours. The alkaloid content of the extract was determined according to the U.S.P. assay procedure for *Belladonna* leaf (*4*).

The new extractor is slightly more efficient than the Soxhlet extractor, especially for short periods of maceration. The difference in alkaloid yield after long periods of maceration is not very great. However, the mixing action produced as the solvent bubbles through the sample is a distinct advantage because it promotes wetting of the sample by the solvent and enhances diffusion (*5*). The absence of an extraction thimble eliminates the possibility that desired constituents will be adsorbed on the thimble. Alkaloid adsorption on the walls of the polyethylene or glass containers has not been observed.

The amount of material which can be extracted conveniently in the Soxhlet-type extractors is limited at any one time by the capacity of the thimble or extraction column. In the new apparatus the size of the container can be varied according to need and can be adapted for both macro- and micro-extractions.

The new extractor has been used efficiently for the ultrasonic extraction of alkaloids from solanaceous plants (*5*). This apparatus should be a useful tool not only for investigating the extent to which sources of energy other than heat can be utilized productively for extracting constituents from plants but also for overcoming many of the technical difficulties in routine extractions (*6*).

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4 January 1963

Diffusion of Gases from Alveolus to Precapillary Arteries

Abstract. *A cardiac catheter with a platinum electrode just proximal to its tip, "wedged" in a branch of the pulmonary artery, was used to demonstrate the appearance of hydrogen or oxygen at the tip of the catheter after inhalation of these gases.*

Prior to 1959 little attention was paid to pulmonary diffusion paths in the lungs aside from the path across the alveolocapillary membrane itself. In 1959 Weibel (*1*) stated that oxygen diffuses from the air spaces into all adjacent tissues. Recent work in this laboratory has shown that transport of gases from

the alveoli through the walls of adjacent small arteries does occur.

Twenty-seven patients, 14 males and 13 females, 4 to 54 years of age (average 23 years) underwent right heart catheterization. In five patients, no hemodynamic abnormality was found. Fourteen with some form of congenital or acquired heart disease had no intracardiac shunt and eight had congenital heart disease with a left to right intracardiac shunt. As part of the procedure, a No. 6F catheter (2.5 mm in diameter), with an annular platinum electrode 2 mm proximal to the tip, was "wedged" in a branch of the right pulmonary artery.

The platinum electrode was used with a potentiometer to sense hydrogen or as a polarograph to sense oxygen (*2*). The electrodes were not calibrated and only relative changes in hydrogen or oxygen concentration at the catheter tip were recorded. The 90 percent response time of the hydrogen electrode is 0.1 second (*3*). The response time of the electrode when sensing oxygen is not known but it appears to have the same order of magnitude as the hydrogen electrode. When hydrogen was used, the patients took a single breath of 100 percent hydrogen and expelled it immediately. When 100 percent oxygen or room air was used, the patients performed one or more respiratory maneuvers designed to change, more or less rapidly, the concentration of the oxygen in the alveoli.

Twenty patients were studied after they had inhaled hydrogen. In 16 the hydrogen was detected by the catheter electrode 0.4 to 0.7 second after it entered the nose, which was recorded simultaneously with a platinum nasal electrode. In four, responses were inadequate for analysis. The observed signals were characterized by an abrupt, very rapid ascent to a smooth peak with a subsequent slower steady return to the base line, not unlike that shown in Fig. 1*a*; the whole sequence was completed in approximately 15 seconds. The responses were the same in all patients.

In eight of these patients, none of whom had a shunt, single breaths of hydrogen taken after the catheter was pulled back to a point 1 to 3 cm proximal to the wedge position produced results identical to those obtained in the wedge position.

When oxygen or room air was used, an increase in the oxygen concentration at the tip of the wedged catheter was detected in less than 1.0 second in 14 out of 18 patients. No change in oxygen concentration was detected in the other four The deflections of the oxygen signal were much greater with 100 percent oxygen than when room air was used. For any one given respiratory maneuver the responses were the same for all patients and no significant differences were noted that could be related to age, sex, or diagnosis. Thus, when a patient took a moderately deep breath of oxygen that was immediately expelled, there was a prompt rise in the oxygen signal to a peak and then a steady, somewhat slower return toward the base line (Fig. 1*a*). If the breath was held the signal after reaching a peak leveled off, and it fell only very slowly as long as the breath was held (Fig. 1*b*). When three successive breaths were taken, three successive rises were recorded with a subsequent return to the base line (Fig. 1*c*).

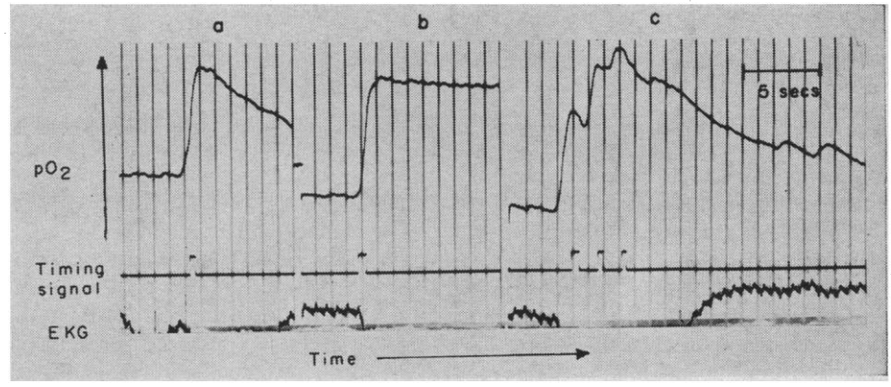

Fig. 1. pO_2 recorded from the tip of a cardiac catheter wedged in a branch of the pulmonary artery during inspiration of 100 percent oxygen: *a*, Single breath immediately expelled; *b*, single breath held; *c*, three successive breaths. Timing signal indicates approximate time of breath(s).

Direct diffusion from the alveoli to the catheter electrode seems the most likely explanation for the results obtained. The observed deflections parallel closely the changes in gas concentration in the alveoli that could be expected under the conditions of the given study. Thus, a single breath of hydrogen produced a transient gradient between the alveoli and the surrounding tissues of as much, probably, as 400 mm-Hg. The hydrogen rapidly diffused along this gradient into adjacent tissues and was detected by the catheter. The recorded signal closely paralleled the changes in concentration which could be expected of the gas in the alveoli. With room air or oxygen, the various respiratory maneuvers employed produced transient increases in the alveolar oxygen concentration above the average alveolar concentration. These changes increased the already existing gradient between the alveoli and adjacent tissues by 50 to 400 mm-Hg, probably depending on the gas used and the respiration performed. Increased amounts of oxygen diffused along this gradient and were detected by the catheter. The recorded signal paralleled expected changes in the alveolar gas concentration.

Staub's work (*4*) with cats' lungs supports the idea of diffusion from the alveoli to the precapillary vessels. He demonstrated oxygenated blood in precapillary arterioles (200 μ in diameter) after exposure of the animals to 100 percent oxygen, but not after exposure to room air. The differences between his observations and those reported here can probably be explained by the differences in vessel size and consequent differences in lengths of the diffusion paths and possibly by differences in sensitivity of detection methods. Thus, the shorter path through the wall of a vessel 200 μ in diameter would permit a greater transport of a gas than would the path through the wall of a vessel 2 mm (2000 μ) in diameter.

Other possible routes of gas transport to the catheter are an arterial path or retrograde passage from the capillary bed. Arterial passage would be too slow to account for the very short appearance times observed here. With the catheter wedged and blocking the vessel, retrograde flow or diffusion fast enough to produce the observed appearance times seems unlikely. The passage of the gas from the alveoli to the catheter must be very fast since the total time required for passage from the nose to the catheter was found to be 0.4 to 0.7 second. Furthermore, if there had been retrograde transport in these studies, there would have been fluctuating signals observed, in time with either the heart beat or the respiratory cycle, neither of which was observed.

No significant differences in the results obtained with the two gases were observed. For technical reasons the hydrogen appearance times were more accurately timed than those for oxygen, but for either gas appearance times are very brief and are of the same order of magnitude.

It is concluded from this study that oxygen and hydrogen can diffuse in detectable amounts directly from the alveoli through the walls of adjacent pulmonary arteries as large as 2 mm in diameter. Of several implications suggested, one of the most interesting is that the diffusion into the arterial walls plays some role in the control of the pulmonary circulation through changes produced in vasomotor activity of the pulmonary arteries.

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14 December 1962

Biosynthesis of Norepinephrine in Isolated Canine Heart

Abstract. *Radioactive dopamine was injected into the blood that was perfusing an isolated heart preparation. Analysis of the norepinephrine obtained from the heart 1 hour later demonstrated that it contained radioactivity. Between 1.4 and 10.8 percent of the norepinephrine present had been formed from the dopamine. The turnover of norepinephrine was substantially higher in the ventricles than in the atria.*

Although the pathway by which norepinephrine and epinephrine are synthesized was first suggested in 1938, it was not until the advent of radioisotopic techniques that this suggestion was confirmed for the adrenal medulla by a number of investigators (*1*). In vitro studies with minced tissue have shown that sympathetic nerves are also capable of synthesizing norepinephrine from either tyrosine or 3,4-dihydroxyphenylalanine (*2*). The heart, among other organs innervated by sympathetic nerves, can extract norepinephrine from the blood (*3*), but it is not known whether the heart is capable of synthesizing norepinephrine from its immediate precursor, dopamine. In our studies we administered radioactive dopamine to an isolated heart preparation and later measured the radioactivity which appeared in the norepinephrine isolated from the heart.

The canine heart preparation we used has already been described in detail (*4*). Oxygenated blood was pumped into the coronary circulation through a cannula inserted into the subclavian artery just proximal to the point at which the descending aorta was ligated. Both venae cavae and the azygous vein were ligated, and the coronary venous return was drained by gravity with a cannula in the right ventricle; the coronary venous blood was then returned to the oxygenator. The circuit of the oxygenator was filled with approximately 1000 ml of blood. Adrenalectomized dogs were used as donors because the levels of catecholamines in dogs subjected to hemorrhage are usually high. We tested the 3,4-dihydroxyphenylethylamine-1-C^{14} hydrobromide, or dopamine (supplied by New England Nuclear Corp.), for isotopic purity by paper chromatography. However, when 20 μc of dopamine were carried through the assay described below, a trace contaminant, the chromatogram of which resembled the chromatogram of norepinephrine, appeared. To eliminate this, the dopamine was further purified by adding 1 mg of nonradioactive norepinephrine and separating the radioactive dopamine by passing the mixture through a Dowex 50 (H^+) column (*5*).

The dopamine-C^{14} (17 μc, 3.10 to 3.26 μc/μmole), in 5 ml of an isotonic saline solution, was administered into the blood entering the coronary arteries during a 30-second period. Perfusion of the isolated heart was continued for the next 60 minutes, and then the heart was removed and frozen immediately in dry ice. This time interval was used because it was judged to be sufficiently long to permit formation of norepinephrine, but well within the time period during which the mechanical activity of the heart was sustained. In experiments 1 and 2 samples of the ventricles, in experiment 3 the entire heart, and in experiments 4, 5, and 6 both atria and ventricles were separated and homogenized with at least five times their volume of 5 percent trichloroacetic acid. The catecholamines in the extract were then adsorbed on aluminum oxide and eluted with 0.2*N* acetic acid by a modification of the procedure of Crout

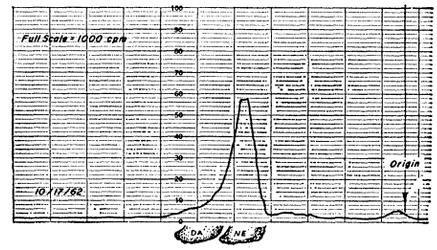

Fig. 1. Radiochromatogram of C^{14}-norepinephrine from isolated, perfused canine heart. (Whatman No. 1 paper; solvent: butanol, acetic acid, and water, 12:3:5). The chromatogram was developed with a ferricyanide spray and is shown together with the scan. The peak of radioactivity corresponds to the norepinephrine spot on the paper chromatogram. Minimal activity appears over the position of dopamine.

et al. (*6*). The eluate was lyophylized, and the residue, redissolved in 3 ml of 0.01*N* HCl, was passed through a column containing Dowex 50 X8 (H^+), 29 mm^2 by 45 mm, with 1.0*N* HCl as the eluent (*5*). Aliquots of the fraction of the eluate corresponding to norepinephrine were assayed for norepinephrine by a fluorometric method (*5*) and for radioactivity by liquid scintillation spectrometry. The isotopic purity of the norepinephrine fraction was demonstrated by ascending paper chromatography (see below). A minimum of 7 μg of norepinephrine was recovered from the chromatography on the Dowex 50 column in all experiments.

In all six experiments radioactivity was incorporated into norepinephrine, with the specific activities ranging from 0.045 to 0.334 μc/μmole (Table 1). In each of the last three experiments, in which the specific activity of norepinephrine in the ventricles was compared with that in the atria, the activity was approximately twice as great in the ventricles. In the four experiments in

Table 1. Specific activity of norepinephrine obtained from isolated canine hearts perfused for 1 hour with 20 μc of C^{14}-labeled dopamine.

Exp.	Specific activity (μc/μmole)			
	Dopamine	Norepinephrine		
		Whole heart	Ventricles	Atria
1	3.26		0.098	
2	3.26		.045	
3	3.10	0.148		
4	3.10	.214*	.236	0.122
5	3.10	.294*	.334	.174
6	3.10	.226*	.254	.138

* Specific activity calculated from the specific activities of the ventricles and atria analyzed individually.