TABLE III. Effect of Addition of Lactose to a Purified Diet on Chick Growth and Bone Formation at the End of 4 Weeks.

	Groups and supplements	Added phosphorus (%)	Lactose (%)	4 wk wt (g)	Bone ash (%)	Availability (%)*
1.	Na_2HPO_4	.4	0	408.5	36.08	
2.	"	"	5	378.1	35.61	
3.	"	"	10	383.3	35.48	
4.	"	"	15	377.1	34.86	
5.	½" + ½ colloidal phosphate	"	0	278.0	33.56	93
6.	*Idem*	"	5	362.5	35.96	101
7.	"	"	10	341.9	35.84	101
8.	"	"	15	351.5	33.16	95

* Availability relative to that of Na_2HPO_4 was determined by comparing bone ash values of groups with corresponding lactose levels when different sources of phosphorus were used.

Summary. The effect of lactose, at levels of 5, 10 and 15% of purified and practical diets, on utilization by chicks of phosphorus from colloidal phosphate was studied. Data presented here demonstrated that utilization of colloidal phosphate by chicks was improved when diets were supplemented with lactose at levels to 10%. The pH of intestinal tract was lowered by lactose supplementation, although this effect was not uniform as lactose level increased.

1. Bergeim, O. J., *Biol. Chem.,* 1926, v70, 35.

2. Robinson, C. S., Huffman, C. F., Manson, M. F., *ibid.,* 1931, v92, 435.

3. Kline, O. L., Keenan, J. A., Elvehjem, C. A., Hart, E. B., *ibid.,* 1932, v98, 121.

4. Motzok, I., Arthur, D., Branion, H. D., *Poultry Sci.,* 1956, v35, 627.

5. Gillis, M. B., Norris, L. C., Heuser, C. F., *J. Nutr.,* 1948, v35, 195.

6. Gillis, M. B., Norris, L. C., and Heuser, C. F., *ibid.,* 1954, v52, 115.

7. Assn. of Official Agri. Chemists, Methods of Analysis, 1950.

8. March, B., Tuckey, R., Biely, J., *Poultry Sci.,* 1958, v37, 405.

9. Beach, J. R., Davis, D. E., *Hilgardia,* 1925, v1, 167.

Received September 28, 1959. P.S.E.B.M., 1959, v102.

Estimation of Cardiac Output by Rapidly Repeated Dye-Dilution Technic. (25377)

DAVID F. OPDYKE AND ROBERT E. SNIFFEN

Dept. of Physiology, Seton Hall College of Medicine and Dentistry, Jersey City, N. J.

A method for estimating cardiac output every 5-8 seconds has been devised. The procedure, based on the indicator dilution principle, has been used in acute experiments where rapidly repeated measurements of cardiac output were required.

Method. A small (Intramedic, PE-100) polyethylene catheter was introduced into the left atrium of anesthetized dogs *via* a pulmonary vein. The small intercostal incision in the fourth left interspace was left open and mild artificial respiration was continued throughout the experiment. The catheter was connected to a specially designed injection valve which controlled the injection of the dye-indicator solution into the left atrium. Time between injections and duration of injection could be pre-determined and phased with other events, such as respiration, by means of electronic controls. A schematic diagram of the injection valve is shown in Fig. 1. The dye-indicator was a tricarbocyanine dye (Cardio-Green®)* prepared as a 0.1% solution. Approximately 0.5 mg (for 10-12 kg

* The Cardio-Green® dye for this project was supplied generously by Hynson, Westcott and Dunning, Baltimore, Md., through the courtesy of Dr. John H. Brewer.

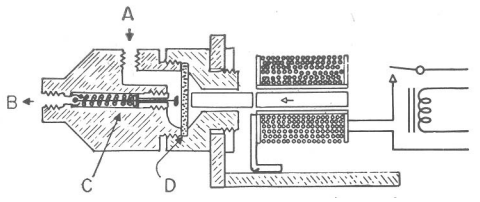

FIG. 1. Dye injection valve. A, inlet from pressurized (600 mm Hg) dye reservoir; B, outlet to dye catheter; C, Standard Schroder tire valve core; D, stiff rubber impregnated fiber diaphragm. Solenoid may be of any type powerful enough to operate valve. Size of assembly is not critical.

dogs) was injected per cycle. Constancy of delivery from the injection valve was established at the beginning of each experiment by collecting in previously weighed vials 10 consecutive deliveries of dye solution. The vials were immediately reweighed before proceeding with the experiment. The results of several typical calibrations are shown in Table I. Arterial blood from an iliac artery (or the descending aorta) was drawn through a Colson densitometer(1) by a specially designed, nonfreezing, constant rate pump. The pump had a capacity of 40 ml which allowed continuous runs to 80 seconds. The densitometer was fitted with appropriate light filters for the dye and its signal output was recorded on an Electronics for Medicine recorder. The densitometer was calibrated at the end of each experiment by making several known dye dilutions in blood and passing these through the densitometer. Carotid artery pressure was measured by means of a Statham P-23AA strain gauge. Coagulation of blood was prevented by liberal use of heparin.

TABLE I. Calibration of Dye Injection Valve, in 10 Consecutive Deliveries.

	June 30, '59	July 6, '59	July 16, '59
	Wt in g		
	.5485	.5540	.5648
	.5496	.5498	.5455
	.5599	.5475	.5600
	.5542	.5491	.5495
	.5539	.5498	.5525
	.5492	.5571	.5634
	.5578	.5523	.5644
	.5459	.5406	.5586
	.5481	.5445	.5599
	.5505	.5455	.5632
Avg	.5517	.5490	.5581
Range	.5459–.5599	.5406–.5571	.5455–.5648
Max error	+2.54%	+2.96%	+3.53%

Results. Fig. 2 shows a continuous record of 7 consecutive optical density curves (fifth from top, Fig. 2) during a period of cardiovascular equilibrium. Dye injections were made precisely 4.1 seconds after onset of lung inflation and, thus, always occurred at the same time during the expiratory pause. Onset of injection is marked on the record both by the injection valve signal (broad pip, top trace, Fig. 2) and by increase of pressure in the injection catheter (third trace from top, Fig. 2). Left atrial pressure fluctuations can be seen on the latter trace. Intratracheal pressure, from which constancy of the respiratory cycle (8.3 sec.) can be checked, is recorded on the second trace from the top of Fig. 2.

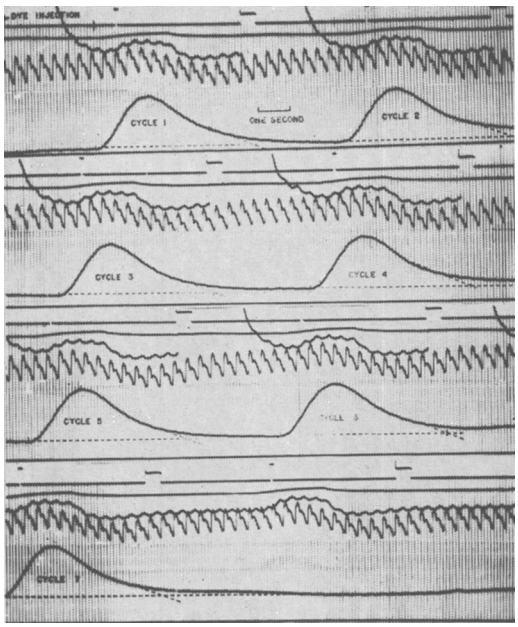

FIG. 2. Continuous record of 7 consecutive dye injections each phased to occur during period of lung deflation in an open chest dog. Top trace, inj. valve signal, narrow pip indicates onset of lung inflation, broad pip marks dye inj. Second trace from top, intratracheal pressure. Third trace, dye inj. catheter pressure (leaves record at moment of inj.). Fourth trace, carotid blood pressure. Fifth trace, dye-dilution curves (continuous, follow to next panel below). Bottom trace, base line.

Due to the relatively small amount of dye injected, and the location of the injection site, recirculation of the dye does not introduce a major difficulty or error in calculation of cardiac output. The major recirculation

TABLE II. Data from 7 Consecutive Dye-Dilution Curves.

	Area (mm^2)	Base line (mm)	Mean ht (mm)	Mean conc. (γ/ml)	Duration of cycle (sec.)	Cardiac output (ml/min.)	Appearance time (sec.)	Foot-peak time (sec)	Peak-ht (mm)
	2300	123	18.8	6.2	5.12	1080	3.30	1.71	39.0
	2560	135	19.0	6.3	5.63	965	3.00	1.79	40.0
	2210	121	18.3	6.2	5.05	1097	3.00	1.67	38.5
	2450	125	19.6	6.5	5.20	1050	3.25	1.67	39.5
	2365	123	19.2	6.3	5.12	1060	3.20	1.71	39.5
	2500	130	19.3	6.4	5.40	994	3.27	1.73	39.5
	2450	132	18.6	6.0	5.50	1035	3.23	1.83	40.0
*	2405	127	18.9	6.2	5.28	1040	3.17	1.73	39.4
†	45.71	2.01	1.67	.01	.26	17.67	.05	.02	.20

* Mean. † Stand. error.

effect may be seen at extreme right end of cycle 7, Fig. 2. However, the descending limbs of the curves do not reach base line before onset of the following curve due to accumulation of dye in the circulating pool and a "tailing off" of curves which seems to be associated with a binding of the dye to glass surfaces of the densitometer cuvette. For these reasons a base line must be constructed for each curve. The point of abrupt increase in optical density is determined and a base line constructed parallel to the record base line (dashed horizontal lines, Fig. 2). The point at which the descending limb should intersect this base line is determined by plotting the slope of the descending limb against time on semilogarithmic paper (see dashed extrapolation lines, Fig. 2) according to the method of Hamilton(2,3). Areas under the curves were measured with a planimeter and the calculation of cardiac outputs made by the classical Stewart formula.

Table II presents results of calculations made on the record shown in Fig. 2. Data on several parameters, in addition to cardiac output, are included for the benefit of those interested in the more theoretical aspects of indicator dilution curves.

Discussion. Under conditions of cardiovascular equilibrium reproducibility of these consecutive dye-dilution curves is impressive. More so, because it was thought probable that mixing of the dye in the left atrium and ventricle would be incomplete. It seems unlikely that results as remarkably consistent as those shown in Table II could be obtained routinely if this is the case. Data from 30 experiments indicate that variation of calculated cardiac output under conditions of cardiovascular equilibrium is no more than $\pm$ 5% which is just about the magnitude of inherent error of the instruments used.

Some precautions are necessary to obtain consistent results. Most important is the necessity of phasing the dye injection with some point in the respiratory cycle, since cardiac output may vary as much as 30% during the respiratory cycle. In 8 experiments cardiac output averaged 20.8% less when the dye injection was made during lung inflation than when the injection was made during the expiratory pause (lung deflated).

This application of the indicator-dilution principle is made possible, of course, by the characteristics of the tricarbocyanine dye(4). Its rapid clearance permits numerous injections without reaching a point of optical saturation. Although the disappearance curve of the dye is steep, no correction has been necessary for continuous runs of less than 80 seconds involving a total injection of about 9 mg of dye. Light transmission characteristics of the dye make fluctuations of arterial oxygen saturation unimportant and permit application of the method to experiments involving asphyxia or marked respiratory changes.

Since quantitative accuracy of the method has not been checked by doing simultaneous Fick determinations, it is not known how the 2 determinations would compare. However, cardiac outputs as measured by the rapidly repeated injection technic are within expected ranges and change in the expected direction and to the expected degree when conditions are changed.

Summary. Injection of 0.5 mg of a tricarbocyanine dye into the left atrium of anesthe-

tized dogs every 5-8 sec. (phased with respiration) results in reproducible dye-dilution curves during cardiovascular equilibrium. The calculated cardiac output does not vary from determination to determination more than ± 5%. The method is useful in the investigation of problems requiring rapidly repeated estimations of cardiac output.

1. Gilford, S. R., Gregg, D. E., Shadle, O. W., Ferguson, T. B., Marzetta, L. A., *Rev. Sci. Instr.*, 1953, v24, 696.

2. Hamilton, W. F., *Am. J. Physiol.*, 1932, v102, 551.

3. Hamilton, W. F., Moore, J. W., Kensman, J. M., Spurling, R. G., *ibid.*, 1932, v99, 534.

4. Fox, I. J., Brooker, L. G. S., Heseltine, D. W., Essex, H. E., Wood, E. H., *Proc. Staff Meet., Mayo Clinic*, 1957, v32, 463, 553.

Received September 30, 1959. P.S.E.B.M., 1959, v102.

Metabolism of Aminonitriles and Related Compounds by the Rat.* (25378)

L. P. MERKOW, S. H. LIPTON, J. J. LALICH AND F. M. STRONG
Depts. of Biochemistry and Pathology, University of Wisconsin, Madison

During our studies on metabolism of β-amino-propionitrile (BAPN), the rat lathyrism factor, considerable variation in severity of symptoms was observed among individual animals(1). The rat eliminated unchanged BAPN in the urine and also a metabolite identified as cyanoacetic acid (CAA)(2). Since the latter compound produced none of the symptoms of BAPN toxicity when fed to weanling rats(3), it presumably is a detoxification product. Using a method for quantitative determination of CAA(4), a study has been carried out on relationship between efficiency of conversion of BAPN into urinary CAA and severity of skeletal deformities. The metabolism of a number of organic nitriles related to BAPN has also been investigated to determine whether other nitriles are likewise detoxified by conversion into CAA. A preliminary account of part of the latter work has been reported(5).

Methods and materials. BAPN was used as the crystalline fumaric acid salt (2 BAPN · 1 fumaric acid), aminoacetonitrile as the hydrogen sulfate acid salt, and 2,2′-iminodipropionitrile (IDPN) as the free base.† The aminonitrile analogs, 4-aminobutyronitrile hydrochloride (mp 144.5-145.5°) and 5-aminovaleronitrile hydrochloride (mp 121-122.5°) were synthesized from corresponding chloronitriles by procedure of Goldberg and Kelley (6). Analysis of 4-aminobutyronitrile hydrochloride. Calc'd for $C_4H_9N_2Cl$: N, 23.23; Cl, 29.40. Found: N, 23.50; Cl, 29.50. Analysis of 5-aminovaleronitrile hydrochloride. Calc'd. for $C_5H_{11}N_2Cl$: N, 20.81; Cl, 26.34. Found: N, 20.84; Cl, 26.70. The intermediate 4-phthalimidobutyronitrile melted at 79-81° instead of 65-66° as reported(6) and the intermediate 5-phthalimidovaleronitrile had mp 77.5-79°. Analysis of 4-phthalimidobutyronitrile. Calc'd. for $C_{12}H_{10}O_2N_2$: N, 13.09. Found: N, 13.90. Analysis of 5-phthalimidovaleronitrile. Calc'd. for $C_{13}H_{12}O_2N_2$: N, 12.27. Found: N, 12.62. Propionitrile, n-butyronitrile, n-valeronitrile, n-hexanenitrile, succinonitrile, and CAA were Eastman products. Ethylene cyanohydrin was furnished by American Cyanamid Co. *Animal experiments.* Sprague-Dawley female rats were used for studies of relationship between urinary CAA levels and severity of skeletal deformities. Older rats weighing 90-300 g were used for study of metabolism of various organic nitriles related to BAPN. The weanling rats (6/group) were fed one of 3 basal diets which differed only in their protein composition (see Table I). Test diets corresponding to the 3 basals were prepared by addition of 3 g of BAPN fumarate to 1 kg of diet. Feeding was *ad lib.* except during

* Published with approval of Director of Wisconsin Agri. Exp. Station. Supported in part by grants from Nat. Inst. Health, P.H.S.

† These compounds were kindly supplied by Abbott Labs., North Chicago, Ill.