Comments. These data support the view that fatty acids released in plasma may be derived from degradation of phospholipids as well as triglycerides(1). The amount of fatty acid released as reported by Dole would require that 4.4 μg of lecithin P per ml be converted to lysolecithin P in 4 hours. The conversion reported herein exceeds this amount slightly; however, an allowance must be made for the conversion of free cholesterol to esterified cholesterol which was not measured.

The conversion of lecithin to lysolecithin on incubating serum is apparently not related to the amount of pancreatic lecithinase A present. Also there seemed to be no difference between pre- and postheparin plasma. Nevertheless the implication of some type of phospholipase A action led us to vary the incubation constituents resulting in the demonstration of a postheparin thermolabile phospholipase A particularly active against egg phosphatidylethanolamine, whose activity is augmented by the presence of protein(8). While we only observed the conversion of lecithin to lysolecithin on incubating serum alone for as long as 20 hours, we have become aware of other conditions under which lecithin may be converted to diglyceride by a phospholipase D produced by a common bacterial contaminant.

Summary. Incubation of human serum or plasma at 38°C for 4 hours results in conversion of approximately 10% of the lecithin to lysolecithin. Serum from patients with acute pancreatitis and postheparin plasma was no different than normal serum or plasma in this respect.

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Received August 20, 1962. P.S.E.B.M., 1962, v111.

In vitro Instability of a Commonly Used Tricarbocyanine Dye. (27848)

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The use of a tricarbocyanine dye (Cardio Green,® Hynson, Westcott, & Dunning) as the indicator for dye dilution measurement of cardiac function has become standard in many laboratories. One of the principal advantages of this dye is its rapid disappearance from the circulation(1), thus permitting serial determination of cardiac output at relatively short intervals. Such serial use of this dye presupposes that it remains stable *in vitro* throughout the study. Fox *et al.*(2) and Miller *et al.*(3) have reported such stability for this dye. However, we were unable to substantiate these findings, and the following study was instituted.

Methods. This study was divided into 2 parts: (1) The stability of Cardio Green dye in the manufacturer's aqueous solvent, and (2) the stability of Cardio Green dye in whole blood.

(1) The stability of Cardio Green dye for 8 hours in the manufacturer's aqueous solvent was investigated from 2 standpoints. The first phase was initiated to see if the wave length of maximum absorbance remained constant, and the second phase to examine the absorbance stability of the dye at a fixed wave length of 775 mμ.

At hourly intervals, an aliquot of the stock dye solution (0.25%, 2.5 mg/cc) was diluted

1:1000 with distilled water and its absorbance spectrum scanned (wave length scan 340 to 900 mμ) on a Beckman DK-2 ratio recording spectrophotometer.* Distilled water in a matched quartz cuvette was used as the reference cell. Prior to the scanning procedure, the instrument was balanced using the same quartz cuvettes filled with distilled water.

This spectrophotometer was also used to study the stability of a 0.25% solution of Cardio Green dye without dilution. The instrument was set for time drive at a wave length of 775 mμ and balanced as previously mentioned. After balancing, Cardio Green dye (2.5 mg/cc) was placed in the sample cuvette with a spacer. The reference cell, containing distilled water and a spacer, was fitted with a gelatinous neutral density filter (Eastman Kodak Co., T = 20%), since it was found that absorbance could not be measured when distilled water alone was used as the reference against a 0.25% solution of Cardio Green dye. Both the reference and sample cells were sealed with paraffin film to prevent evaporation during the study period.

A pair of copper constantine electrodes was placed inside the sample compartment and another set of electrodes was exposed to the room environment. Simultaneous temperatures were recorded continuously on a multi-channel Minneapolis Honeywell Model Y-153X62P6-X-16 (V) recorder (range 0 to 50°C).

Two approaches were taken to studying the absorbance stability of the 0.25% solutions of Cardio Green dye. In one instance, the sample and reference cells were placed within the sample compartment of the spectrophotometer and studied continuously for 8 hours after preparation of the dye solution. To eliminate the possible effects of the slightly elevated sample compartment temperature upon the dye solution, the dye and reference cells were introduced into the sample compartment for the readings only and were allowed to stand at room temperature during the 30-minute intervals between readings.

The stability of the Beckman DK-2 ratio recording spectrophotometer was proven in the following manner. A spectral transmission standard of reagent grade copper sulfate ($CuSO_4 \cdot 5\ H_2O$, Baker) prepared in accordance with National Bureau of Standards specifications(4) was placed in the sample cuvette with a spacer. A matched quartz cuvette containing distilled water and fitted with a neutral density filter (T = 20%) was used as the reference. Both cuvettes were sealed with a paraffin film to prevent evaporation. The spectrophotometer was set at a wave length of 775 mμ and placed on time drive. The standard was studied continuously for 8 hours. Again, sample compartment and room temperature were concomitantly recorded. The absorbance of the spectral transmission standard remained constant during this 8-hour time interval, demonstrating absolute stability of the spectrophotometer which was used in this study.

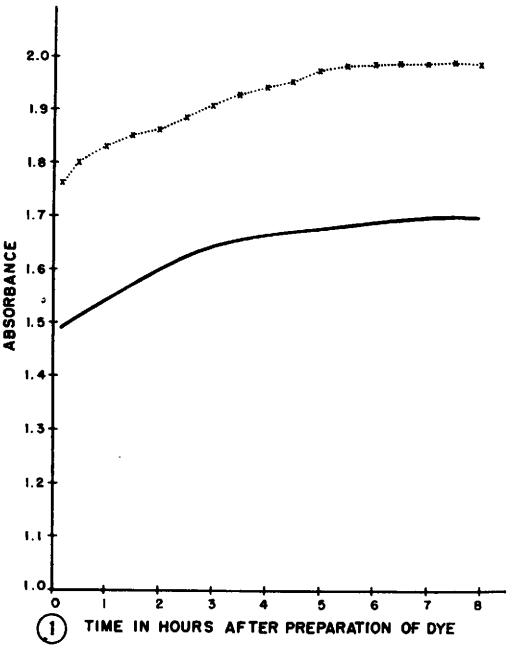
(2) The stability of Cardio Green dye in whole blood was studied using a Waters model XC-250A densitometer cuvette (with F CG filter) and model XP-250A densitometer control. At hourly intervals for 8 hours, known concentrations of Cardio Green dye in whole blood(5) were drawn through the densitometer cuvette with a Gilford model 105S constant flow system and the deflections recorded on a Sargent model MR high impedence recorder. In this manner, changes in absorbance of various concentrations of dye in whole blood were observed with the passage of time.

Results. Spectrum scan (340 to 900 mμ) of a 1:1000 aqueous dilution of a 0.25% solution of Cardio Green dye showed that the maximum peak absorbance of the dye was at 775 mμ. This is in direct agreement with the reports of other investigators(2). The wave length of peak absorbance for this dye did not deviate from 775 mμ during the first 8 hours after preparation, despite the fact that optical density varied.

The absorbance of the dye in the manufacturer's aqueous solvent and in whole blood

* Whenever this spectrophotometer was used, it was allowed to stabilize for a minimum period of 3 hr prior to use.

was observed to increase progressively during the study period. The continuous line in Fig. 1 depicts the changes in absorbance of Cardio Green dye (0.25% aqueous solution) at a wave length of 775 mμ during a continuous 8-hour study period. The temperature of the sample compartment varied between 34.8°C and 37.2°C, whereas the room temperature was somewhat lower (range 23°C to 28°C). The broken line in Fig. 1 represents changes in absorbance of a different sample of Cardio Green dye (0.25% solution) which remained at room temperature during the 8-hour examination period. Each point (×) represents the absorbance recorded at that particular time.



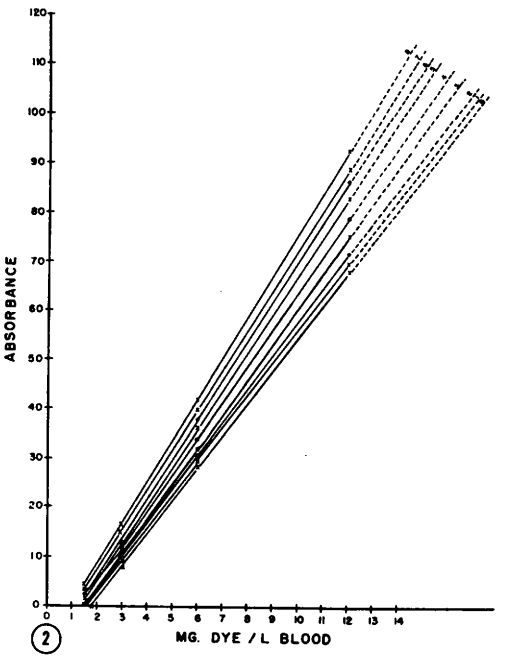


FIG. 1. Changes in absorbance of 0.25% aqueous solution of Cardio Green dye during first 8 hr after preparation. The continuous line depicts changes in absorbance of a 0.25% aqueous solution of Cardio Green dye during a continuous 8-hr study period (wave length 775 mμ). Broken line represents changes in absorbance of a 0.25% solution of Cardio Green dye which remained at room temperature during 8-hr examination period (wave length 775 mμ). Each point (×) represents absorbance recorded at that particular time interval.

FIG. 2. Changes in absorbance of various concentrations of Cardio Green dye in whole blood during 8 hr. Concentration of dye in whole blood (mg/l) is depicted along abscissa. Relative units of absorbance (in terms of mm deflection on the Sargent recorder) are seen on the ordinate. Time in hr after mixing the dye with whole blood is diagonally inscribed on the dotted extension of concentration absorbance lines.

Due to the similarity between the curves in Fig. 1, the effect of an elevated sample compartment temperature can be disregarded as the prime factor in the instability of the dye.

Fig. 2 depicts changes in absorbance of various concentrations of the Cardio Green dye in whole blood as a function of time. Even though absorbance of the dye changes, it is apparent from the linearity of each slope that the dye behaves in accordance with the Beer-Lambert Law(6) at each time interval after preparation.

Discussion. Our data have failed to confirm the reports of others(2,3) on the stability of Cardio Green dye in distilled water and in whole blood. The data presented here clearly show that Cardio Green dye changes appreciably in absorbance throughout a study time of 8 hours.

To illustrate the magnitude of error which could be introduced into serial determinations of cardiac output, the following example was taken from experimental data previously reported from this laboratory(7). A series of cardiac output determinations(8) was made at hourly intervals on an anesthetized mon-

TABLE I. Effect of *in vitro* Instability of a Commonly Used Tricarbocyanine Dye (Cardio Green) on Calculation of Cardiac Output (C.O.).

Time (hr) after preparation of dye in aqueous solvent (2.5 mg/cc)	Cardiac output (ml/min)		
	Corrected calibration curve	Original calibration curve	% difference
0	2308	2308	0
1	1923	1852	– 4
2	1613	1389	–12
3	1875	1224	–35
4	2000	1786	–11
5	1613	1470	– 9
6	1852	1613	–13
7	2055	1744	–15

grel dog. A dye calibration curve was prepared at the time of each determination(5) using the same vial of diluted dye. In Table I is shown the cardiac output at each time interval as calculated by the appropriate calibration curve (Column I) and that as calculated using only the original (o) calibration curve (Column II). In Column III is tabulated the percentage error which would have occurred had not a calibration curve been constructed at the time of each cardiac output determination. In this experiment a falsely low cardiac output of 4 to 35% below the correct cardiac output would have been obtained if only the initial calibration curve had been used. The diminished cardiac output in Column II compared to that in Column I for that particular time interval reflects instability of the dye only.

Summary. The use of an indicator dye for serial determinations of cardiac function by the dye dilution technic requires *in vitro* stability of the dye for the duration of the study period, since repeated calibration curves are not routinely performed. The light absorption of a commonly used tricarbocyanine dye (Cardio Green) in the manufacturer's solvent and in whole blood was studied continuously (Beckman DK-2 spectrophotometer) for 8 hours following preparation of the dye solution. Although the wave length of peak absorbance in aqueous solution did not deviate from 775 millimicrons, a progressive increase in absorbance (as much as 10% in 8 hours) was consistently observed, in direct contrast to previous reports. However, throughout the period of study, the dye continued to conform to the Beer-Lambert Law, thus permitting correction of the changing absorbance by construction of a calibration curve each time the dye is used. Unless such a correction is made, a significant error can be introduced into serial estimations of cardiac output.

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Received August 29, 1962. P.S.E.B.M., 1962, v111.