

3. When hypertensive rats were adrenalectomized and maintained on small doses of cortisol, there followed a decrease in blood pressure and restoration of level of pressor material in the unclamped kidney. 4. It is suggested that diminution of pressor material in the unclamped kidney is not a prerequisite but a consequence of the pathological situa-

tion leading to renal hypertension.

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Use of Dyes with Rapid Bloodstream Clearance for Serial Determination of Cardiac Output. (24038)

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The T-1824 dye dilution method has been widely used for measurement of the cardiac output. Because of albumin binding the dye leaves the bloodstream very slowly. This causes serious technical and clinical difficulties when repeated injections of the dye are given for serial estimation of the cardiac output, for example, appearance of bluish coloration of skin and subcutaneous tissues, and development of excessively high concentrations of dye in the plasma above the range of accurate determination with a spectrophotometer. These facts suggested that a dye with rapid bloodstream clearance might be more suitable than T-1824 dye for frequent serial determination of cardiac output. The present study is concerned with investigation from this viewpoint of a group of 45 blue dyes.

Materials and methods. Healthy dogs weighing 10 to 25 kg were fasted 48 hours before each experiment. Anesthesia was produced by intraperitoneal injection of sodium pentobarbital (30 mg/kg body weight), after which an endotracheal tube with inflatable cuff was placed in the trachea. The dyes dissolved in distilled water and sterilized by heating were injected (0.25 mg/kg body weight) into one exposed femoral vein. Venous blood samples were removed from the opposite femoral vein before injection and at intervals after, *viz.* 1, 5, 10, 15, 20, 25, 30, 45, 60, 90, and 120 minutes. Optical density readings of

dye-plasma samples were made at point of maximum spectral absorption with Beckman Spectrophotometer, DU model. Spectral absorption curves and disappearance rates of 38 of the 45 blue dyes were investigated. The remaining 7 blue dyes were either partially soluble in water (Acetin Blue) or insoluble (Alcian Blue, Sudan Blue, Ultramarine Blue, Oil Blue, Galamine Blue, Alazarin Blue S) and were, therefore, unsuitable for further study. In the cardiac output experiments, the selected dyes were injected (0.15 mg/kg or 0.25 mg/kg body weight) into one femoral vein. Following injection, arterial blood samples were collected (from a polyethylene tube inserted through one femoral artery into the aorta) at 2-second intervals into cuvettes of motor-driven sample collector. Cardiac output values were calculated from formula of Stewart(1), of Hamilton and associates(2) as modified by Nicholson and Wood(3).

Results. *Dyes cleared within one min.* The following dyes were cleared from the bloodstream in less than one minute; Anthracene Blue, Toluidin Blue O, Victoria Blue R, Night Blue, Poirrer Blue, Prussian Blue, Luxon Fast Blue MBS, Niagara Blue 2B, Delphine Blue, Isamin Blue, Cyanin Blue G, Fast Acid Blue, Brilliant Cresyl Blue, Brilliant Alazarin Blue, and Azure Blue B.

Dyes cleared within 5 min. The following dyes were cleared between one and 5 minutes; Acid Alazarin Blue 2B, Nile Blue, Toluylene

TABLE I. Dyes Cleared from Bloodstream within 120 Min.

Dye		Max spectral absorption, $m\mu$	Time of complete clearance from bloodstream, min.
Patent	Blue A	640	< 25
Brilliant	"	630	"
Methylene	"	670	< 20
Chicago	" B	580	< 15
Diamond	" 3B	520	< 10
Thymol	"	440	< 10
New Methylene	" N	580	< 15
Oxamine	" 4R	550	< 60
Trypan	"	600	< 120
Azo	"	560	"
Patent	" V	640	"

Blue, Rhoduline Blue 6G, Celestine Blue, Alazarin Blue GS, Aniline Blue WS, Dianil Blue 2R, and Victoria Blue.

Dyes cleared within 120 min. Four dyes in this group (Table I) have maximum spectral absorption of 630 $m\mu$ or above, namely Patent Blue A, Patent Blue V, Brilliant Blue, and Methylene Blue. The plasma optical density readings produced by injection of Patent Blue A, Patent Blue V, and Brilliant Blue were much higher than those produced by equal amounts of Methylene Blue. Accordingly, we used Patent Blue A, Patent Blue V, and Brilliant Blue for determination of cardiac output.

Dyes cleared after 120 min. Because of their slow clearance from the bloodstream the following dyes were considered unsuitable for repeated injection at short intervals of time: Direct Sky Blue, T-1824, and Niagara Sky Blue.

Comparative values for cardiac output. Values for cardiac output obtained in dogs with selected dyes are comparable (Table II). Despite the fact that the paired determina-

tions were not performed simultaneously, the estimated values are close.

Discussion. The following criteria were used in selecting dyes most suitable for repeated injection into the bloodstream for purposes of serial determination of cardiac output: (1) The dye should have a bloodstream clearance rate of approximately 20 to 30 minutes. However, dyes with longer clearance rates up to 120 minutes may be used. (2) The dye should reach and maintain a high concentration in the blood during the first minute after injection. (3) The maximum spectral absorption of the dye should be higher than that of hemoglobin, preferably, 630 $m\mu$ or above. In this way, hemolysis in a plasma dye sample will produce minimal interference with spectrophotometric determination of dye concentration. In addition, it is essential to establish that the selected dye is non-toxic and that solutions of the dye obey Beer's Law. Too rapid clearance of a dye from the bloodstream may not permit an adequate build-up of dye concentration to occur during the initial period when cardiac output is being determined. Of the 38 dyes studied, only 3 fulfilled the criteria listed above.

Summary and conclusions. Bloodstream clearance rates and spectral absorption curves of 38 blue dyes have been investigated. The results suggest that suitable dyes for frequent serial estimation of the cardiac output are Patent Blue A, Brilliant Blue, and Patent Blue V. These dyes leave the bloodstream rapidly after injection; they have an adequate build-up of concentration during the first minute; and their maximum spectral absorption occurs at wave lengths of 630 $m\mu$ or higher. Cardiac output values obtained in dogs with Patent Blue A, Brilliant Blue, Patent Blue V,

TABLE II. Comparison of Cardiac Output Values Obtained by Use of Patent Blue A, Patent Blue V, Brilliant Blue, and T-1824 Dyes.

Dog wt, kg	First cardiac output		Second cardiac output (30 min. later)		
	cc/min.		cc/min.		
		mg/kg			mg/kg
13.6	3090	Patent Blue A .25	2840	T-1824	.25
13.2	2752	Brilliant Blue .15	2993	T-1824	.15
11.8	3068	Patent Blue A .15	3024	Patent Blue V	.15

and T-1824 dyes are presented and compared.

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Foreign-Body Type Reaction in Fat Cells.* (24039)

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During studies of the response of rats to diets containing very high amounts of saturated fats, a foreign-body type reaction was observed in fat cells of adipose tissue adjacent to sections of various organs prepared for histological examination. This report describes these altered fat cells and various dietary conditions under which they occur. Although a similar abnormality had been observed earlier with one of these fats by Cox and DeEds,[†] their data have not been published.

Methods and materials. Weanling male and female rats were obtained from Holtzman Rat Co. and fed one of the following diets. Each diet contained 20% vitamin-test casein, 4% B-vitaminized casein(1), 5% USP XIV salts, 4% corn oil, 17% glucose, and supplements of Vit. A, D, and E at levels of 40,000 I.U., 4,000 I.U., and 510 I.U./kg diet, respectively. The saturated fats incorporated at a level of 50% were fully-hydrogenated lard, palmitic acid, stearic acid, or a saturated partially acetylated monoglyceride(1). Ethyl stearate was fed at 45% with 5% cellulose added to provide body to the diet. A control group was given 50% lard. Rats were sacrificed at 8-week intervals and examined grossly. Portions of perigastric, perirenal, periadrenal, perigonadal, and subcutaneous

fat were removed from each rat, fixed with Bouin's solution, sectioned, and stained with hematoxylin and eosin.

Results. Gross examination of the viscera and body cavities was not remarkable except for general fullness of the gastrointestinal tract with creamy colored food and an occasional mottled appearance of fat depots in animals fed the saturated fats.

Microscopic examination of sections showed an unusual foreign-body type reaction in fat from rats fed saturated fats (Fig. 1, top). The lesion consisted of a multinucleated cellular reaction usually located within the fat cell membrane with nuclei arranged evenly about periphery of the fat cell or in clumps. In many instances, the material which occupied the center of the fat cell, appeared to have been partially dissolved, leaving delicate slit-like spaces which were radially oriented and suggestive of fatty acid crystals (Fig. 1, bottom). The reactive process usually involved 2 or more adjacent fat cells and was separated from similar areas by uninvolved fat cells. No acute inflammatory reaction, evidence of recent hemorrhage, or birefringent material were associated with the fat reaction.

The phenomenon occurred within 8 weeks in rats fed diets containing palmitic acid, stearic acid, ethyl stearate, or saturated partially acetylated monoglycerides and within 16 weeks in rats fed the fully-hydrogenated lard diet, but was not observed in rats fed the control lard diet. The flecks of opaque white material observed grossly in the depot fat of some rats were associated with the severest occurrence of the foreign-body type reaction as seen microscopically.

* Communication No. 247 from Research Labs., Distillation Products Industries.

[†] Observed in rats fed saturated acetylated monoglycerides for long periods of time by A. J. Cox, Dept. of Pathology, Stanford Univ., Calif., and F. DeEds, Western Regional Research Laboratory, Albany, Calif., private communication. *Added in proof:* Recently published by Cox and DeEds, *Am. J. Path.*, 1958, v34, 263.