propagated in capuchin kidney cells remained virulent, in the doses inoculated, after 4 or 5 serial tissue culture passages. As shown in Table III, paralytic disease was produced after intracerebral inoculation. In addition, the affinity of the virus for the CNS was demonstrated after intraspinal inoculation. When the virus was injected into the lumbar area, it multiplied there and frequently ascended the cord; this manifested itself by leg paralysis followed by arm paralysis.

Summary. Cultures of trypsinized kidneys of South American capuchin monkeys (*Cebus capucina*) supported the multiplication of Type 1 polioviruses, even though no cytopathic changes were observed by microscopic examination of the cultures. The failure to observe cytologic changes was found to be due to the small proportion of cells in the culture capable of supporting the growth of virus. After 4 to 5 tissue culture passages the viruses remained virulent for the monkey brain and spinal cord. No evidence was obtained for the propagation of Type 2 and 3 strains in the capuchin cultures.

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Received October 5, 1955. P.S.E.B.M., 1955, v90.

Measure of Irritation by Quantitative Determination of Extravasated Dye.* (22099)

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Intravenously administered colloidal dyes have been observed to concentrate at sites of inflammation(1-5). This phenomenon has been the basis for tests to measure the intensity of irritation(6-10). In such tests, visual estimations of the quantity of dye at irritated sites were graded by various systems, either direct or by comparison to standard color charts. The results of visual observations in this manner are influenced by subjective bias and are not easily reproducible.

This report describes a method for the quantitative removal of accumulated Evans blue dye from irritated tissue and its measurement spectrophotometrically. This should be a reliable index of the degree of irritation, provided the amount of extravasated dye is proportional to the extent of irritation. A modification of the method of Caster, Simon, and Armstrong(11,12) for the measurement of plasma volume was used for extracting the dye from tissue.

Methods and materials. White rats, (Sprague-Dawley), 115-130 g, were anesthetized with an intraperitoneal injection of sodium pentobarbital. The skin on the posterior side of the hind legs was slit to expose the gastrocnemius muscles and 0.03 cc of the test material was injected into each muscle using a ¼ inch, 27 gauge needle. Fifteen minutes after the injection of the test material,

* The authors wish to thank M. L. Pabst for his advice and O. S. Carpenter for advice on statistical treatments.

0.3 cc of 1% Evans blue in saline was injected intravenously in the tail vein. Thirty minutes later the animal was sacrificed, both gastrocnemius muscles removed and placed in a semi-micro Monel blendor jar containing 15 cc benzalkonium chloride (Zephiran Chloride, 12.8%). The tissue and Zephiran Cl were blended at high speed in a 2-speed Waring blendor for 2 minutes, 13 cc isobutyl alcohol were added, and blending was continued for 30 seconds on low speed. The mixture was transferred to a small glass-stoppered reaction tube and centrifuged at 1750 r.p.m. for 3 minutes. After centrifugation, 3 layers were visible: a top layer of isobutyl alcohol containing the dye, a middle layer of white macerated tissue, and an aqueous-Zephiran Cl bottom layer.† Using vacuum, the 2 bottom layers were aspirated and discarded. Approximately 3.5 g sodium sulfate anhydrous were added to the remaining isobutyl layer to remove excess water and the tube was shaken by hand for one minute. It was centrifuged at 1750 r.p.m. for 5 minutes, and the supernatant decanted into a cuvette. Absorbency readings were made at 620 mμ in a Coleman Junior spectrophotometer. By means of a standard dye curve, the absorbency readings were converted into micrograms (μg) of dye.

Experimental. To ascertain the extent of dye recovery from the muscle tissue, the 3 layers of the first centrifugation were examined. Spectrophotometric analysis of the aqueous layer indicated that no dye was present. Microscopic examination of the macerated tissue layer showed no trace of the dye. Spectrophotometric analysis showed that the Evans blue was contained in the isobutyl layer. Therefore, this indicated quantitative recovery of the dye. The standard dye curve was prepared by processing increments of Evans blue, 10 to 150 μg in Zephiran Cl, according to the preceding method. The curve followed Beer's law confirming the work of Caster, *et al.*(13); therefore, it was used to convert the absorbency readings of all tissue extractions to μg of dye. The amount of dye and extraneous components extracted from non-irritated muscles after intravenous injection of Evans blue was determined as a "blank." The average blank for 22 rats was equivalent to 24.0 μg of dye. Because extraneous tissue components contributed to this value, the absorbency readings for normal muscles and test material injected muscles with dye absent were compared and found to be in agreement. Therefore, the blank was subtracted from all results obtained on the test materials to give a corrected value. Control injections of physiological saline and sterile water were tested and the results given in Table I. Physiological saline injections gave no evidence of irritation. Sterile water for injection was very slightly irritating which agrees with the findings of Weatherby(7).

† NOTE: Occasionally, contact of Zephiran Cl with the blendor assembly produced a blue color in the aqueous-Zephiran layer. A test for copper in this layer was positive. The blue color was insoluble in isobutyl alcohol, therefore, did not interfere with absorbency readings of the dye.

TABLE I. Dye Measurements for the Blank and Control Injected Rats.

Materials tested	No. of rats	Dye value, avg μg/rat	Corrected dye value, avg μg/rat
Blank	22	24.0	—
Saline	16	23.7	−.3
Sterile water for inj.	6	27.9	3.9

Results. To test the procedure for graded responses to a known irritant, a series of concentrations of NF IX Formaldehyde were prepared and tested. The results are given in Table II. A regression line was calculated from the data in Table II and is shown in Fig. 1. Calculations of the regression line showed Slope $= 27.1$; Stand. Dev. $= \sqrt{107.16} =$

TABLE II. Dye Measurements for Formaldehyde Concentrations.

Material tested	No. of rats	Corrected dye value, avg μg/rat
Formaldehyde 1.6 %	8	43.5
.8	8	39.9
.4	18	26.3
.2	10	18.7
.1	10	6.8
.05	10	7.1

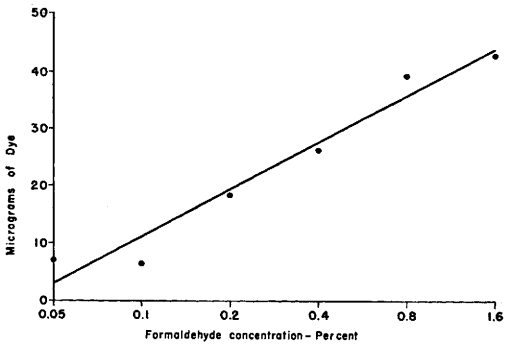

FIG. 1. Regression line for formaldehyde series.

10.352. The average dye measurements did not deviate from the regression line by more than could be attributed to experimental error. The slope of the regression line relating dye concentrations to dose concentrations was highly significant. Therefore, the method provides a quantitative measurement of degrees of irritation, assuming the degree of irritation to be proportional to the concentration of the irritant.

Preliminary examination was made of various categories of injectables to observe a range of irritation. The samples were tested at concentrations normally employed clinically. The results on materials examined to date are given in Table III.

TABLE III. Dye Measurements for Injectables.

Materials tested	Concentration	No. of rats	Corrected dye value, avg μg/rat
Preservatives			
Phenol	.5 %	3	−.9
Chlorobutanol	.5	3	1.6
Methylparaben	.2	3	2.2
Propylparaben	.02	3	1.9
Vehicles			
Cottonseed oil	—	4	4.1
Peanut oil	—	4	−.6
Al. Monostearate w/peanut oil gel	2.0 %	4	−.2
Local anesthetics			
Procaine HCl	2.0 %	8	3.4
Piperocaine HCl	2.0	7	1.4
Lidocaine HCl	2.0	8	4.3
Vitamin products			
Liver inj. (crude)	2 μg B_{12}/cc	8	11.1
Idem (refined)	10	11	5.3
" "	20	8	4.1
Nicotinic acid amide	10.0 %	4	5.6
Calcium compounds			
Ca. Gluconate	10.0 %	23	14.4
Ca. Chloride	2.5	13	35.9
Idem	1.25	10	24.0

The results in Table III indicate the preservatives, vehicles, and local anesthetics tested were only slightly irritating at clinical concentrations. The liver injection products did not cause irritation proportional to the amount of cyanocobalamin present; however, it appeared that refinement of the product reduced its irritating properties. Calcium chloride, which is not recommended for intramuscular injection due to its irritating properties, showed the highest degree of irritation in our study. Concentrations of 5.0 and 10.0% produced average dye values of 53.2 and 54.6 μg respectively. Because a significant increase was found in extracted extraneous components due to the irritant injection alone, these values were omitted from Table III. Dilutions of the calcium chloride from ¼ to ⅛ the clinical strength showed more irritation than calcium gluconate, which also had a high order of irritation.

Because of the current interest in anti-inflammatory compounds, a preliminary study was made to determine the adaptability of this method to measure anti-inflammatory activity. The procedure was modified only in that the test animals were injected with the anti-inflammatory material previous to the injection of the irritant, Calcium Gluconate, 10.0%. Intraperitoneal injections of Solu-Cortef‡ (hydrocortisone sodium succinate), 5 mg/rat, were used to obtain the anti-inflammatory effect. The preliminary results showed that a single dose injected 1 to 5 hours before the irritant would lower the amount of dye recovered by about 25%. This indicated that the method may be modified to measure quantitatively anti-inflammatory activity.

A preliminary test of xylene treatment of the rat abdominal skin indicated that a modification of the method may be used to measure quantitatively the irritation caused by agents applied topically.

Discussion. It appears that our preliminary results cover the range from non-irritating

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to highly irritating products. After the accumulation of more data, an attempt will be made to describe irritation on a unitage basis. At present, however, it is recommended that comparisons of irritation be made within categories of compounds, since there seems to be no universal agreement on a standard irritant. The same practice would probably apply to studies of the anti-inflammatory compounds.

In the course of this investigation, alternate sites of injection and modifications of the procedure have been used. The rat ear, rat testes, rat abdominal skin, and the longitudinal back muscle of the rabbit have been used successfully as an injection site for the irritant. In each case, the procedure of dye removal was modified slightly, but essentially the method remained the same.

Summary. An objective quantitative method to measure the degree of irritation has been described. It has been used to obtain a valid dose-response curve on a known irritant, formaldehyde, and to measure the irritating properties of various categories of injectables. The method has been modified to permit measurement of anti-inflammatory activity and topical irritation.

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Received October 20, 1955. P.S.E.B.M., 1955, v90.

Effect of Cyanide on Growth and Respiration in Earle's Strain L Cells.* (22100)

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Respiration in most tissues is inhibited by 3×10^{-3}M cyanide(3,8,12,14). Cyanide has been used very little as an inhibitor of cells grown *in vitro*. Danes and Leinfelder(2) noted that 0.1×10^{-4} to $.38 \times 10^{-4}$ M HCN inhibited growth of chick embryo tissue 73%. Experiments reported below were designed primarily to ascertain to what extent Earle's strain L cells, which have been grown in culture for more than 12 years(4), are cyanide sensitive.

Materials and methods. Strain L cells were obtained through the generosity of Dr. Wilton R. Earle and were cultured on glass (D-3.5 Carrel flasks) in Earle's solution consisting of 40% balanced saline, 40% horse serum and 20% chick embryo extract (in Earle's saline solution, 1:1)(4,5). The culture medium was renewed 3 times weekly and cultures gassed with 5% CO_2 in air. In experiments to test the effect of cyanide on growth, a cell suspension was obtained by gentle agitation of stock cultures. The cells were concentrated by centrifugation at 1000 rpm(7), resuspended in fresh medium after

* This investigation was supported (in part) by a research grant from the National Cancer Institute, of National Institutes of Health, Public Health Service.

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