consequences from emotional stimulation of the sympathetic pathways in the many diseases thought to be psychosomatic.

Summary. Stimulation of the cervical sympathetic trunk, application of local epinephrin, and injection of epinephrin intravenously has been found to produce, not only the classical picture of decreased circulation from narrowing of the vascular bed, but also what looks like blood "sludge" within the vessels. It is suggested that this may be of interest to those studying small vessel circulation and "sludge" and may have applications in general surgery, general medicine, and psychosomatics.

The author was assisted in these studies by Drs. Robert D. O'Malley, William A. Jarrett, and Octa C. Leigh.

Received October 25, 1949. P.S.E.B.M., 1949, v72.

Quantitative Measurement of Cutaneous Fluorescein Fluorescence as Indicator of the Capillary Circulation.* (17510)

ALFRED A. SCHILLER

From the Department of Physiology, University of Illinois College of Medicine, Chicago, Ill.

When sodium fluorescein is administered intravenously, it rapidly enters the interstitial fluid compartment. Due to its strong green fluorescence, its presence can be readily detected even in low concentrations when the skin, mucosa, or the surface of organs are viewed in suitably filtered long wave UV in a darkened room. Recently the passage of fluorescein into the interstitial fluid spaces was described as a function of capillary permeability.(1,2) However, no conclusive experiments designed to demonstrate this relationship have appeared in the literature. Although the specific factors regulating the passage of the dye through the capillary wall are not known with certainty, it can be clearly demonstrated that the patterns of cutaneous fluorescence, with respect to time and intensity, strongly mirror the capillary status of the region observed. Thus, intracutaneous epinephrine prevents fluorescein fluorescence at the site of injection, whereas histamine, acetylcholine, heat, etc. augment fluorescence.(1-4) Further, the intensity of fluorescence is quantitatively related to the concentration of the intracutaneously administered drugs as well as the amount of intravenously administered dye.(1,3) On the basis of this cursory relationship between fluorescein fluorescence and vascularity, it seemed worthwhile to refine methods of accurately measuring fluorescence *in vivo* with the intention of providing an objective tool for the quantitative study of the capillary circulation in physiologically intact preparations. Although Lange and Krewer(5) and Crismon and Fuhrman(6) have measured cutaneous fluorescein fluorescence by quantitative methods, experience with components of their apparatus have shown that they lack sufficient sensitivity and control to warrant usefulness as precision detectors of minute changes in fluorescence.

The instrumentation and methods reported herein are a modification of a procedure used for the determination of fluorescence in solids,(7) operating in principle similar to that of the dermofluorometer.(5)

Description of the Apparatus. The fluorescence measuring device designated as the

* Supported by a grant in aid from the Graduate School Research Board.

1. Bukantz, S. C., and Dammin, G. J., *Science*, 1948, v107, 224.

2. Lange, K., and Boyd, L. J., *Arch. Int. Med.*, 1944, v74, 175.

3. Schiller, A. A., *Fed. Proc.*, 1949, v8, 139.

4. Schiller, A. A., to be published.

5. Lange, K., and Krewer, S. E., *J. Lab. and Clin. Med.*, 1943, v28, 1946.

6. Crismon, J. M., and Fuhrman, F. A., *J. Clin. Invest.*, 1947, v26, 259.

7. Schiller, A. A., and Cecchini, L. P., Project NM 001 008, Report No. 3, 21 July, 1948, Naval Medical Research Institute, Bethesda, Maryland.

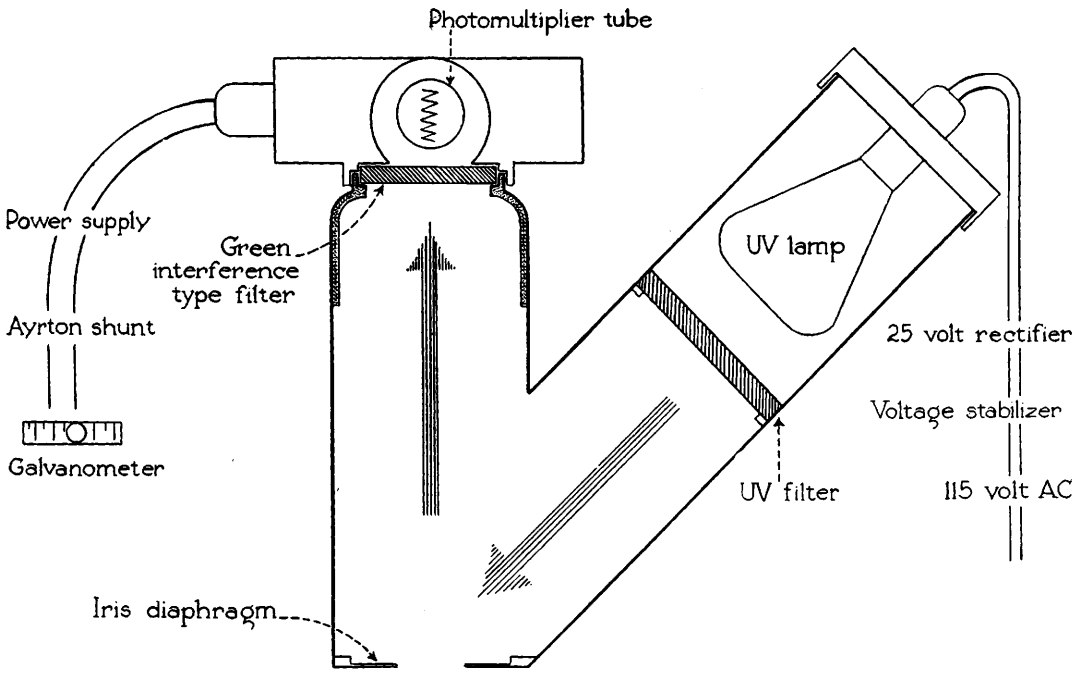

FIG. 1.
Fluorescence measuring device. (Not drawn to scale).

fluoro-illuminometer (F-I) consists essentially of a metal "Y" tube which serves as a rigid holder for a UV light source, photocell and filters (Fig. 1). The light source is a small 4 watt mercury vapor lamp† operating on stabilized rectified current of 20-24 volts. A Corning No. 5874 filter secured beneath the lamp absorbs the visible and transmits the UV radiation between λ 310-410 mμ, with a peak transmission at λ 365 mμ. The transmitted UV activates the specimen surface to fluorescence. The emitted fluorescence and an undetermined fraction of reflected UV passes normal to the specimen surface through an interference type filter‡ which functions as a monochromator, transmitting a band of green light which peaks at λ 525 mμ. Thus, only the fraction of radiation from the specimen surface corresponding to the peak emission of fluorescein fluorescence reaches the multiplier phototube.§ The photo current is read from a sensitive spotlight galvanometer.‖ An Ayrton shunt¶ included in the circuit permits the utilization of the full galvanometer scale over wide fluctuations of fluorescence intensity without altering phototube sensitivity. The high sensitivity of the photomultiplier tube more than compensates for the rather great transmission loss (60-70%) by the interference filter. Standardization of the apparatus is accomplished by the use of a stable reference material consisting of a 2″ x 2″ piece of green fluorescent glass** seated in a close fitting holder over a thin sheet of polished aluminum foil (Fig. 2). A primary

† General Electric, 360 BL RP12.

‡ Half width = 15±5 mμ; Farrand Optical Co., Inc., New York City.

§ RCA 1P21.

‖ Rubicon: Sensitivity = .002 μa/mm; Resistance = 4800 ohms; CDRX = 110,000 ohms. The phototube and housing, power supply, control unit and galvanometer were purchased as an integrated unit from Farrand Optical Co., Inc., New York City.

¶ Leeds and Northrup No. 2164, 100,000 ohms.

** Canary glass No. 3750, Corning Glass Works, Corning, N.Y.

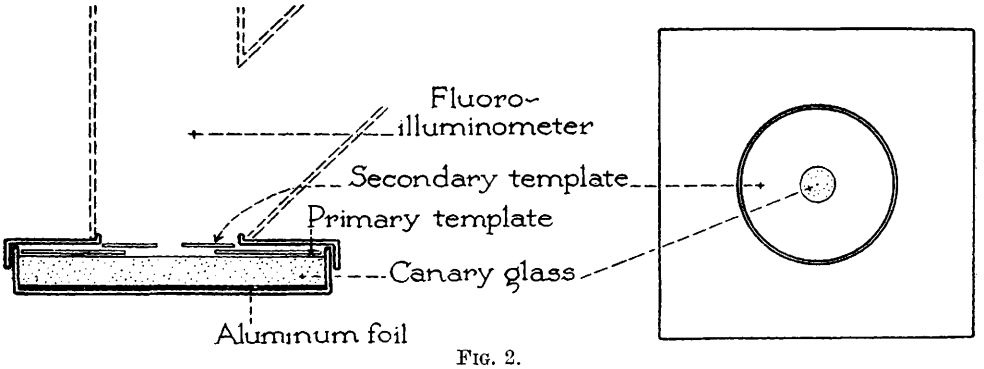

FIG. 2.
Reference standard. (Not drawn to scale).

template of black heavy paper with a central aperture 18 mm in diameter covers the entire upper surface of the canary glass. A metal cover with a 4.6 cm diameter lipped aperture fits snugly over the reference holder and primary template to accommodate the base of the F-I in a light-tight gravity seal. A number of circular secondary templates having central apertures from 1-10 mm in diameter are cut from thin copper sheeting and coated with dull black lacquer. These discs have a slightly smaller diameter than the aperture in the metal reference standard lid so that they may be easily removed by simple inversion of the reference holder.

Procedure. The UV lamp in the fluoro-illuminometer is started approximately 10 minutes prior to the use of the instrument to ensure thermal stability and constancy of emission. The F-I is placed over the lipped aperture of the reference standard after insertion of a selected secondary template.†† The phototube is zeroed for dark current and the sensitivity is adjusted to read 100% on the galvanometer scale. Prior to the administration of the fluorescein, the F-I is placed over the skin sites the fluorescence of which is to be measured and pre-dye readings are made which are later subtracted from each post-dye determination, thus compensating for inherent skin fluorescence. After each measurement, or after a series of consecutive measurements, the F-I is again seated on the reference standard. A difference of more than 3% between the initial and final reference readings invalidates the measurement. If care is taken to ground and shield the equipment, and to select a UV lamp with stable characteristics, a series of 6 consecutive readings can be made in approximately one minute with reference variations of ± 1%. If the fluorescence from a small specific skin site, *e.g.* an intradermal wheal, is to be measured a blackened paper template about 2″ x 2″ with a central aperture corresponding to the diameter of the wheal is placed on the skin and the F-I centered on it. For more accurate centering on a discrete area, or when fluorescence is to be expressed as intensity per unit area, a prism finder head and iris diaphragm calibrated in terms of diameter can be fitted to the F-I. An accurate objective measurement of the circulation time of the dye is easily obtained by leaving the F-I on the skin while the dye is being injected. A sudden fluctuation of the galvanometer string indicates the exact moment of arrival of the dye at the skin site.

Results and discussion. The cutaneous vascular responses to a variety of chemical and physical agents employing the above procedure is under investigation. In most instances the vasoactive drug is prepared in serial dilutions and injected intradermally in the depilated abdomen of the rabbit, followed by the intravenous injection of sodium fluorescein (25 mg/kg in 5% aqueous solution). Fluorescent intensity over these sites

†† The template selected is determined by trial and error. When the correct secondary template is chosen, it will usually be found that the entire range of fluorescence encountered can be read on the galvanometer scale without changing the shunt or phototube sensitivity.

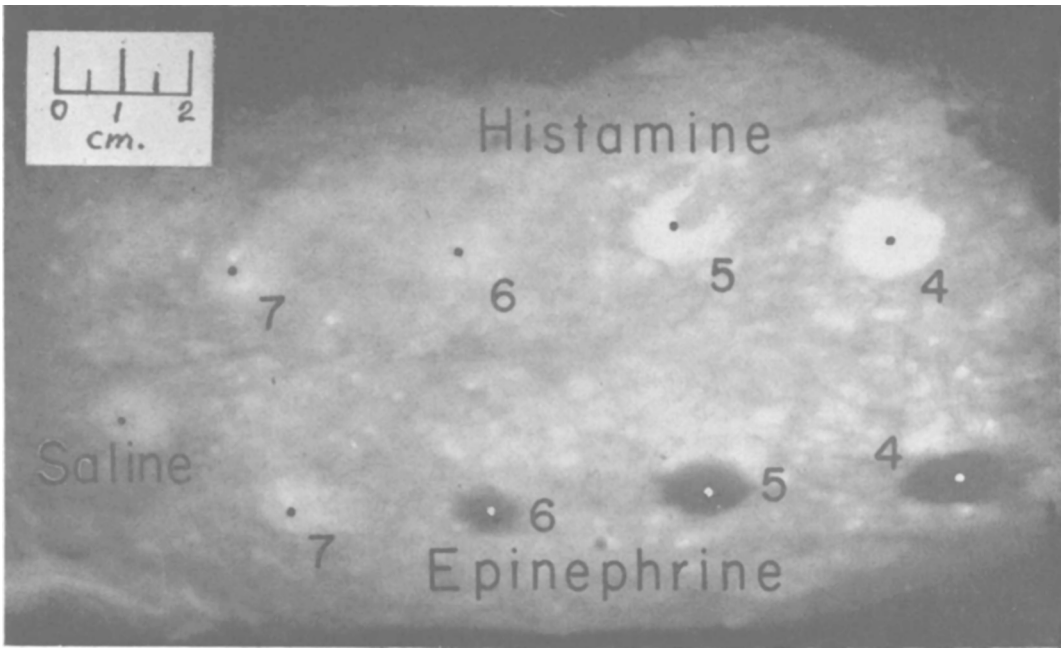

FIG. 3.
UV photograph of whealed rabbit's abdomen 9 min. after i.v. fluorescein (*cf.* Fig 4). The numbers refer to the concentrations of the drugs, *viz.*, 10^{-4}, 10^{-5}, etc. The black and white dots are artefacts to aid in identifying the center of each wheal.

is found to vary in a regular manner depending on the specific activity and the amount of the drug used as well as the anatomical location of the wheal. (Fig. 3). Time-intensity curves of fluorescein fluorescence are shown (Fig. 4) in which histamine and epinephrine were employed. It will be seen that generally the largest amount of epinephrine and histamine show the least and highest fluorescent intensities respectively, which is consistent with the known activities of these drugs. Also, the several dilutions of either drug produce a readily differentiable response at some point along the time axis which permits quantitative inter-comparison. The opaque colloidal dye, trypan blue, which is reputed to be an indicator of capillary permeability but which is poorly quantitated, has been compared with fluorescein in parallel experiments.(4) In every instance in which an agent has produced a positive trypan blue response, the intensity of cutaneous fluorescein fluorescence was well above the control level. The same agents in dilutions not capable of producing a positive trypan blue response did, however, show a relative increase in fluorescein intensity, implying that the fluorescein test is a sensitive indicator for intense as well as mild stimuli. It was noted frequently that agents which produced a positive trypan blue response, *viz*: concentrated histamine, heat, topical bromobenzene in ether, etc., were unphysiological stimuli and may have injured the capillary endothelium, suggesting that such a test may be invalid as an indicator of permeability changes in the physiological range.

Accepting the presently held hypothesis that trypan blue is an indicator of increased capillary permeability, and that fluorescein behaves similarly in that both dyes appear extravascularly in greater concentrations under identical experimental conditions, it may be deduced that the intensity of cutaneous fluorescein fluorescence may be dependent to some extent upon permeability changes. It is evident that a rigorous experimental demonstration must be provided before a permeability factor can be read into the cutaneous fluorescein intensity measurements in its pres-

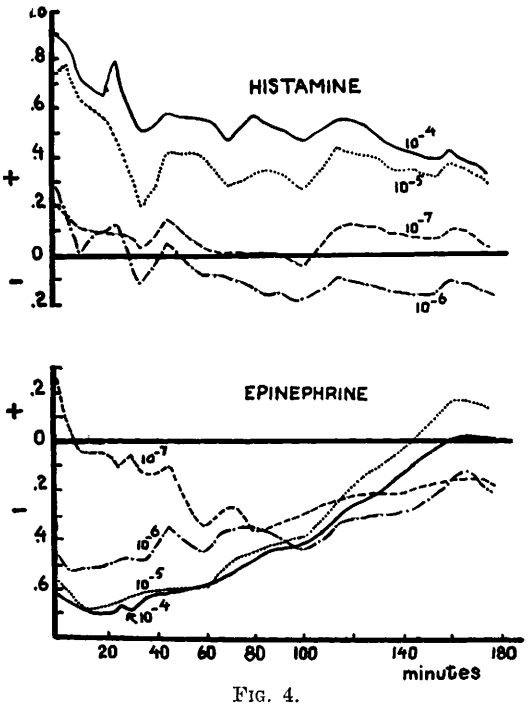

FIG. 4.

Fluorescein fluorescence time-intensity curves plotted as differences from the control saline wheal. Ordinate $= \frac{\text{fluorescence of drug wheal}}{\text{fluorescence of saline wheal}}$. + = relative hyperfluorescence; — = relative hypofluorescence.

ent form. The rate of disappearance of fluorescein from the blood and the intact normal skin of the rabbit are closely parallel,(4,6) indicating that the altered time-intensity dye curves produced by the administration of vasoactive drugs must be a property of altered capillary hemodynamics and/or a change in the permeability of the capillary wall. The exact factors responsible for the rate of dye exchange through the capillary wall can only be determined by the simultaneous measurement of these independent variables. The estimation of cutaneous fluorescein fluorescence as described above quantitatively indicates the gross rate of dye exchange across the capillary wall (neglecting lymphatic participation), but does not offer insight regarding the responsible specific factor(s).

Summary. 1. A quantitative method for measuring cutaneous fluorescein fluorescence is described.

2. The intensity of cutaneous fluorescein fluorescence is shown to parallel the expected capillary responses to various concentrations of vasoactive drugs.

3. The procedure described can be used to obtain in physiologically intact, unanesthetized experimental animals and probably human beings, a quantitative, sensitive and objective estimate of the cutaneous capillary circulation under a variety of experimental conditions.

The author acknowledges his indebtedness to Mr. Carl Griesmeyer for the skillful and intelligent construction of the apparatus described in this paper.

Received October 25, 1949. P.S.E.B.M., 1949, v72.

Role of Vitamin B_{12} in Reproduction of Poultry.* (17511)

ROBERT J. LILLIE, M. W. OLSEN, AND H. R. BIRD

From the Bureau of Animal Industry, U. S. Department of Agriculture, Beltsville, Md.

Research of the past few years showed that dried cow manure and certain animal protein supplements contained an unknown factor (or factors) required by chickens for growth and reproduction.(1,2) When crystalline B_{12} became available, it was found to possess activity for chick growth equivalent to that of the unknown growth factor occurring in cow manure and liver extract.(3) The results

* Presented as part of symposium on vitamin B_{12} and related factors, 116th meeting of American Chemical Society, September 22, 1949.

1. Rubin, M., and Bird, H. R., *J. Biol. Chem.*, 1946, v163, 387.

2. Whitson, D., Titus, H. W., and Bird, H. R., *Poultry Sci.*, 1946, v25, 143.