A system is being developed to reduce the data. The raw data will be recovered from magnetic tapes, and a very compact record will be provided by a two-channel, direct-writing oscillograph if the fetal heart rate falls within predetermined "normal" limits. If these are exceeded, the time base of the writing system will be automatically increased, and a more detailed record will be secured. At the same time, the information will also be digitized and plotted by an $x-y$ recorder.

It is hoped that the use of modern instrumentation methods may aid in the elucidation of clinical fetal distress.

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References and Notes

1. M. Cremer, *Münch. med. Wochschr.* 53, 811 (1906).
2. This study is being supported by research grants from the Medical Fluid Research Fund of Yale University, the Association for the Aid of Crippled Children, and the National Heart Institute, National Institutes of Health, U.S. Public Health Service (grant No. H-2272). We are indebted to C. L. Buxton, professor and chairman of the department of obstetrics and gynecology, to W. Watson, chairman of the department of physics, and to Andrew Patterson, Jr., department of physical chemistry, for many helpful suggestions and guidance in this study. We wish to thank the Burdick Corp. of Milton, Wis., for providing us with the two-channel Elema electrocardiograph and Tektronix, Inc., Bronxville, N.Y. for their cooperation with the instrumental aspects of this study.
* Markle scholar in medical science.

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Intraocular Arterial Homotransplants for Studying Atherosclerotic Lesion Regression

The purpose of this report (*1*) is to introduce the use of intraocular arterial homotransplantation for studying spontaneous or experimentally induced regression of specific atherosclerotic lesions in healthy animals. The operative procedures used were modifications of those employed by several investigators (*2*). A portion of an artery is excised aseptically and atraumatically from an anesthetized donor animal, rinsed with warm (37°C) mammalian Ringer's solution, and opened longitudinally with sharp scissors. Square pieces of approximately equal size are cut from selected normal or uniformly atherosclerosed areas. One piece is retained as a control for histologic study and for comparison with transplants removed later in the course of the experiments. The others are placed in warm Ringer's solution until they are transplanted into the host animals.

Each of the anesthetized host animals is taped securely to an animal board to minimize reflex head movements during the operation. Three drops of 0.5-percent tetracaine hydrochloride solution are applied topically to the eye into which a transplant will be made. The sclera is grasped firmly with fixation forceps, and a 3- to 4-mm incision is made through the cornea, near the corneoscleral junction, with a cataract knife. While one edge of the cut cornea is gently lifted with sharp-pointed forceps, a corner of one of the transplants is grasped with slender mouse-toothed forceps and gently inserted through the incision into the anterior eye chamber. A slender blunt instrument (strabismus hook) is used to slide the transplant across the anterior eye chamber to the opposite side and wedge it there between the cornea and iris with the intimal surface facing outward. Finally, a small amount of penicillin ointment is applied to the operated eye. Depending on the experimental plan, transplants of either normal or atherosclerotic arteries may be made into one or both eyes of each host.

Several groups of animals may be prepared. For some experiments, healthy young litter-mate animals of the same sex should comprise an experimental group and serve as hosts for arterial transplants taken from a litter-mate of the same sex. For other experiments, animals of the same or opposite sex from another litter of the same species and strain could be used. In either case, the donor may or may not have been subjected to atherogenic procedures.

At selected times (for example, every 3 months), a host animal of each group can be sacrificed, and histologic sections of the normal and atherosclerotic transplants can be prepared by the same methods as those used for the control pieces. The sections of control and transplanted pieces from each group can then be studied to determine the nature and degree of any structural or chemical changes, or both, which may have occurred in the transplants, either spontaneously or as the result of experimental procedures on the host. It may also be possible to determine the order in which each of several changes occurs. Comparison of the results obtained using dogs, rats, or other resistant species with those obtained from rabbits and other susceptible species may reveal some of the reasons for species differences in susceptibility to experimental atherosclerosis.

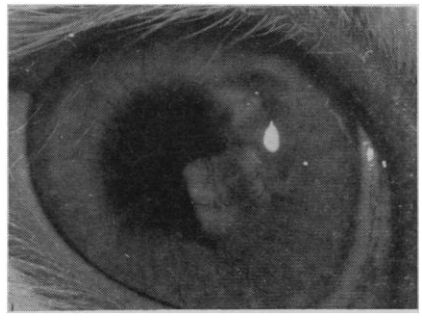

Fig. 1. Homotransplant of atherosclerotic aorta in the anterior eye chamber of a young female rabbit, 4 weeks postoperatively. Note the extensive invasion of the thickened intima by blood vessels from the iris.

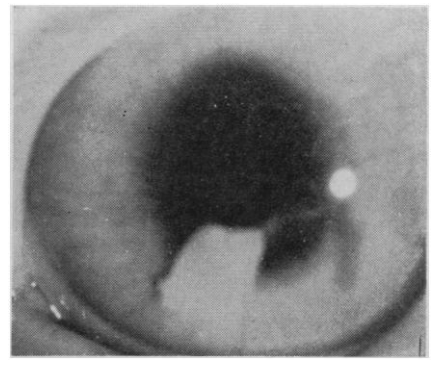

Fig. 2. Homotransplant of normal aorta in the anterior eye chamber of a young female rabbit, 4 weeks postoperatively. No blood vessels invading the intima can be observed.

In experiments with rabbits, recovery of the host animals from the operation is prompt and is not complicated by infection. The adventitia of all the normal and atherosclerotic transplants becomes attached to the anterior surface of the host's iris by fibroconnective tissue in less than 8 days. Within 30 days, the adventitia of all the transplants is invaded by several clearly visible blood vessels from the iris. In addition, the thickened intima of all atherosclerotic transplants becomes extensively vascularized (Fig. 1), but that of the normal transplants does not (Fig. 2).

The transplants of normal aorta have been in place for 6 months, and those of atherosclerotic aorta for 6½ months. It has not been determined how much longer than this they will persist, but apparently there will be sufficient time to permit long-term studies of the effects of drugs, diets, and other experimental regimens on the structure and blood supply of the transplants. These test animals should be more responsive to drugs and diets that may cause regression of atheromata than animals which have been subjected to rigorous atherogenic procedures. Should certain procedures be found to accelerate regression of the transplanted lesions, their application to the treatment of human atherosclerosis is indicated.

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References and Notes

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2. H. S. N. Greene and J. A. Saxton, Jr., *J. Exptl. Med.* 67, 691 (1938); C. D. Turner, *Anat. Record* 73, 145 (1939); J. E. Markee, *Carnegie Inst. Wash. Publ. No. 518*, 219 (1940); P. N. Martinovitch, *Methods Med. Research* 4, 240 (1951); G. M. Ramm, *J. Exptl. Zool.* 130, 107 (1955).

3 January 1957

Excitation Lifetime of Photosynthetic Pigments in vitro and in vivo

To measure the fluorescence decay of pigments (*1*) with excitation life-times of the order of 10^{-9} sec, we constructed an electronic instrument that contained no Kerr cells or piezoelectric cells (to avoid unsolved problems of quantitative interpretation of the data). A hydrogen flash lamp (*2*) supplied a light pulse lasting less than 1 mμsec. The fluorescence induced by this flash was measured with a photomultiplier; the signal was applied directly to the plates of an oscilloscope, and the display was photographed. Figure 1 shows the recordings of the lamp flash (*A*) and of a fluorescence flash (*B*). The time constant of the instrument (which accounts for most of the width of the upper curve) is of the same order of magnitude as that of the fluorescence decay; the two effects were separated by mathematical analysis, presuming the decay to be exponential.

The fluorescence lifetime τ of several pigments was determined *in vitro* in this way with a precision of ± 7 percent, and —for the first time—also *in vivo*, with a precision of ± 20 percent. The most important results are given in Table 1.

The quantum yield of fluorescence (φ) can be derived from the measured excitation lifetime τ and the "natural" lifetime τ_0 [calculated by integration of absorption curves; see Lewis and Kasha (*3*) and Förster (*4*)] by means of the well-known equations $\tau = \varphi\tau_0$. In the case of pigments *in vitro*, the results can be compared with the quantum yields measured by Forster (*5*) and by Latimer (*6*); good agreement was obtained with the results of the latter.

A wide difference appeared, however, between the fluorescence yield ($\varphi \simeq 10$ percent) calculated from the lifetime of chlorophyll fluorescence *in vivo* (assuming τ_0 to be the same as *in vitro*!) and that determined by Latimer (2 to 3 percent). One possible interpretation of this discrepancy is to assume two forms of chlorophyll *in vivo* [a hypothesis for which some spectroscopic evidence has been obtained by other investigators (*7*)]; the fluorescent form must then account for about one fourth of the total, and the nonfluorescent form for about three-fourths of the total. An alternative is to attribute the discrepancy to different conditions of the experiment—more specifically, to associate the higher value (10 percent) with the "dark-adapted," and the lower value (2 to 3 percent) with the "light-adapted" state of the cells.

With red algae, we were able to observe a difference in the rise of phycoerythrin—sensitized, as compared with that of direct chlorophyll fluorescence (Fig. 2). The former was delayed by about 0.5 mμsec—in approximate agreement with the value one can calculate from the life-time of phycobilin excitation *in vitro* and the efficiency of energy transfer from the phycobilins to chlorophyll *in vivo* as estimated by Duysens (*8*).

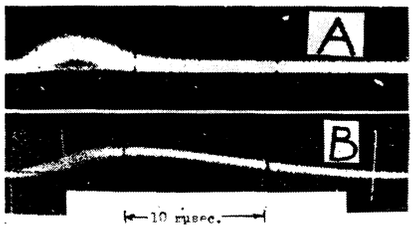

Fig. 1. Typical cathode-ray tube displays. Curve *A*, response of the instrument to the lamp flash; curve *B*, fluorescence of chlorophyll *a* in methanol.

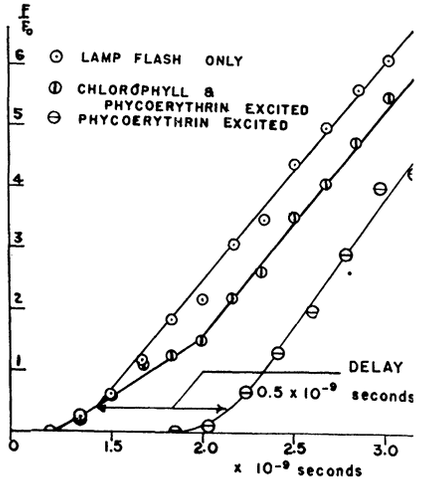

Fig 2. Onset of direct and sensitized fluorescence.

Table 1. Fluorescence lifetime of pigments. The accuracy of the measured lifetimes varies from ± 0.4 to ± 0.5 mμsec.

Material	τ (mμsec)	τ_0 (mμsec)	$\varphi = \tau/\tau_0$
Chlorophyll *a*			
in benzene	7.8		
in ethyl ether	5.1	15.2*	0.33†
in methanol	6.9		
Chlorophyll *b*			
in benzene	6.3		
in ethyl ether	3.9	23.0*	0.17†
in methanol	5.9		
Chlorophyll *a*			
in *Chlorella*	1.6	15.2*	0.11†
in *Porphyridium*	1.5	15.2*	0.10†
in *Anacystis*	1.2	15.2*	0.08†
Methyl chlorophyllide (*a* + *b*)			
in benzene	6.7		
in ethyl ether	4.8		
in methanol	6.5		
Phycoerythrin in phosphate buffer (*p*H 6.0–6.2)	7.1	8.3‡	0.85§
Phycocyanin in phosphate buffer (*p*H 6.0–6.2)	1.8	3.0‡	0.53§
Fluorescein	4.8	5.3‡	0.91§
Eosin Y	1.6		

* Natural lifetime was determined by integrating the absorption band of chlorophyll in ethyl ether.
† Fluorescence yields were calculated from lifetimes; they agree well with the direct determinations of Latimer (*6*) in solution and are about 4 times higher *in vivo*.
§ Fluorescence yields were determined by Latimer (*6*).
‡ Natural lifetime was determined from measured lifetime and fluorescence yield.

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References and Notes

1. This work was carried out with the assistance of the Office of Naval Research.
2. The lamp was developed by John Malmberg of the University of Illinois Betatron Laboratory.
3. G. N. Lewis and M. Kasha, *J. Am. Chem Soc.* 67, 944 (1945).
4. T. Förster, *Fluoreszenz Organischer Verbindungen* (Vandenhoek and Ruprecht, Göttingen, 1951).
5. L. Forster, thesis, University of Minnesota (1951).
6. P. Latimer, thesis, University of Illinois (1956); P. Latimer, T. T. Bannister, E. Rabinowitch, *Science* 124, 585 (1956).
7. E. Rabinowitch, *Photosynthesis and Related Processes* (Interscience, New York, 1956), Vol. II. 2, pp. 1752–1754, 1847.
8. L. Duysens, thesis, University of Utrecht (1953).

17 December 1956