averaging all results from both cores, we obtain an estimate of 0.03 disintegration/ sec cm<sup>2</sup> contained in the sediment column. This is of the expected order of magnitude. No more can be said at present. Even this is not without interest from the point of view of the constancy of the cosmic-ray flux.

In addition to the data given here, other measurements were made to identify the observed activity. Samples G-2 and G-4 were combined, and a second complete cycle of purification was carried through. The original samples contained  $0.92 \pm 0.06$  disintegration/min (assuming that the self-absorption correction of Be10 applies). The recycled sample showed  $0.68 \pm 0.20$  disintegration/min after correction for chemical yield. Seven months elapsed between the two measurements.

An absorption curve was run on a composite sample of H-1, H-2, H-4, and H-5, using polyethylene absorbers. The data are plotted in Fig. 1. A curve for a synthetic sample of Be10 under the same conditions is shown for comparison. The absorption curve in close cylindrical geometry approaches an exponential (7). The half-thickness of the natural sample is  $17 \pm 4$  mg/cm<sup>2</sup>, while that of the synthetic sample is  $21.2 \pm 0.3 \text{ mg/cm}^2$  in the same region of the absorption curve. Using Libby's relation for half-thickness versus energy and mass number (8), we obtain  $E = 0.52 \pm 0.08$  Mev, compared with 0.56 Mev for the known activity. A further check on the half-thickness is the self-absorption correction of the composite sample. The count rate of the composite sample was  $0.65 \pm 0.07$  times the sum of the original samples, while the calculated value is 0.76. No gamma activity was found in any sample.

One set of data has been discardedthat obtained in my effort in Chicago to measure the absorption curve of the original G composite. The data indicate strongly that absorbers or other materials were contaminated.

Our intention is to use Be<sup>10</sup> if possible for radioactive age determination. Much work on the geochemistry of beryllium still must be done before this method can be safely used.

A final word should be said on measurement techniques. A counting method appears to be the most practical at the present time, although Peters (4) has suggested a photographic-plate technique. It is worth noting that, if the beryllium content of sediments is of the order of 1 ppm, the  $Be^{10}/Be^9$  ratio is about  $10^{-7}$ . This does not seem to be permanently outside the range of solidsource mass spectrometry, although the difficulties would be extreme.

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# **Quantum Yields of Fluorescence** of Plant Pigments

The fate of excitation energy in photosynthesis requires elucidation. Toward this end, we have determined the quantum yields of fluorescence of several photosynthetic pigments, in vitro and in vivo (1). The measurements were made with a specially constructed integrating sphere (2). The main results are shown in Tables 1 and 2.

Our values of the quantum yields of fluorescence  $\phi$  of chlorophylls *a* and *b* are about 40 percent higher than those reported by Forster (3). We believe that this difference is the result of a more accurate determination in our experiments of the detector sensitivity as a function of wavelength, and a more reliable "sampling" of the incident light and fluorescence with the sphere. Both the ratio of the fluorescence yield of chlorophyll b to that of chlorophyll aand the strong effect of solvent on the former, reported by Forster, are confirmed.

As was anticipated by Duysens (4),  $\phi$ of chlorophyll in vivo was found to be an order of magnitude higher than the value previously accepted on the basis of the measurements of Wassink et al. (5). The new data indicate that the actual lifetime of excitation of the first excited singlet state of chlorophyll a in vivo is of the order of 10<sup>-10</sup> second, rather than 10<sup>-11</sup> second, as has been previously assumed. This offers a correspondingly better chance for migration of excitation energy between chlorophyll molecules.

The quantum yield of chlorophyll fluorescence in vivo was previously known to change (usually to increase but sometimes to decrease) with increasing intensity of the exciting light at photosynthesis-saturating intensities (6). We found this yield to vary also with the exciting intensity when the latter was as low as 0.01 of that required for the compensation of respiration by photosynthesis (see Fig. 1). Recent measurements by Brugger (7) are consistent with the results shown in Fig. 1.

Franck's theory of "narcotization" of the chlorophyll complex, which could explain the intensity dependence of  $\phi$  at

Table	1.	Quar	ntum	yields	of	fluorescence
of pig	mer	nts in	solut	ion.		

Pigment and solvent	Wave- length of exciting light (mµ)	Quantum yield of fluores- cence* ( $\phi$ )
Chlorophyll a		
Ethvl ether	430	0.33
Methanol	436	0.32
Pyridine	436	0.35
Ethyl chlorophyllide a	ļ	
Ethyl ether	436	0.33
Chlorophyll b		
Ethyl ether	436	0.16
Methanol	436	0.084
Phycocyanin (from		
Synechocystis sp.) Water (0.1M phos- phate buffer, pH 6.2 Phycoerythrin (from Porphyridium	) 546	0.53
cruentum)		
Water (0.1 <i>M</i> phos-		
phate buffer, pH 6.2	) 480	0.85
Fluorescein		
Aqueous NaOH	436	0.91

\* Corrected for self-absorption of fluorescence by extrapolation to zero concentration.

Table 2. Quantum yields of fluorescence of pigments in the living cell.

Wave- length of exciting light (mµ)	Quantum yield of fluores- cence* (\$\$
osa	
436	$0.027^{+}$
436	0.017-
	0.020
436	0.028†
436	<b>~</b> 0.015†
546	∼ 0.030-
	0.025
	$Wave-length of exciting light (m\mu)$

\* Corrected for self-absorption of fluorescence by extrapolation to zero concentration. † Excited with 50 erg/cm<sup>2</sup> sec.

t Extrapolated to very low intensities of exciting light.



Fig. 1. Quantum yield of fluorescence of Chlorella cells. Yield is shown as a function of the logarithm of the intensity of the exciting beam (averaged over its path in the vessel).  $\lambda_{ex.} = 436$  mµ. The same values plotted on a linear rather than semilogarithmic scale lead to a curve that is concave downward rather than upward.

photosynthesis-saturating intensities, is not applicable at the low intensities studied here. Apparently, the intensity dependence of  $\phi$  in vivo is due to two (or more) factors that come into play in different intensity ranges. The change in  $\phi$  near or below the compensation point may reflect the participation in photosynthesis of respiratory intermediates whose relative importance must decrease as the intensity increases.

We did not observe an inflection in the  $\phi = f(I)$  curve corresponding to the one reported by Kok (8) for photosynthesis. But kinetic considerations show that, even if different factors govern  $\phi$  primarily in different intensity regions, such inflections would not necessarily occur.

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## Sperm Transport in the **Reproductive Tract of** the Female Rabbit

Previous estimates of the time required for sperm ascent in the reproductive tract of the female rabbit usually have been based on flushing various regions of the tract at definite intervals after mating (1, 2). The present note offers another approach to the problem-namely, tubal ligation at various times after mating and the subsequent examination of the trapped eggs for evidence of fertilization. While this work was in progress, a paper using the same technique with several variations appeared (3); the results reported here confirm Adams' findings

Mature New Zealand giant white does were used in the experiments (4). The rabbits were bred once to males of proved fertility. At intervals of 0.5, 2, 3, 4, and 5 hours post coitum, laparotomies were performed, and the fallopian tubes were doubly ligated and sectioned at the uterotubal junction. The rabbits were killed between 48 and 52 hours post coitum, the tubes were flushed with 0.9 percent saline solution, and the recovered eggs were then examined for evidence of normal cleavage and development.

The results indicate that sufficient sperm are in the tubes of every animal by 5 hours post coitum to fertilize all viable eggs (Table 1). The increase in the percentage of fertilized eggs between 4 and 5 hours post coitum parallels a similar rise in the number of sperm recovered from the tubes during the same time span (2). However, it is misleading to account for the increased percentage of fertilized eggs on the basis of an increase in the mean number of spermatozoa. The most likely explanation for the increased percentage of fertilized ova is that sperm have reached the tubal level of every animal by 5 hours post coitum. Before this time, there is considerable individual variation in the rate of sperm

Table 1. Fertilizing ability of rabbit sperm in ligated fallopian tubes. (Eggs examined 48 to 52 hours post coitum).

			-		•
Time		No.	No.	Per-	No. of
post	No.	of	of	cent-	ani-
coitum	of	follic-	eggs	age of	mals
tubes	rab-	ular	re-	ferti-	with
ligated	bits	rup-	cov-	lized	ferti-
(hr)		ture	ered	eggs	lized
		points			ova
1/2	3	21	20	0	0
2	5	48	31	19	1
3	5	54	53	47	3
4	5	29	25	40	3
5	5	46	41	98	5

entry into the tubes as measured by the number of animals with fertilized ova at different hours post coitum (Table 1).

Differences from animal to animal in uterine motility and in the mechanical barrier offered by the cervix and uterotubal junction probably account for the variability in the rate of sperm transport before 5 hours post coitum (2, 5). GILBERT S. GREENWALD\*

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# **Cold-Adapted Genetic**

### Variants of Polio Viruses

Variants of the polio viruses have been obtained through passage in various in vitro systems. Enders, Weller, and Robbins (1) passaged the Brunhilde strain (antigenic type I) in tissue cultures of human embryonic skin muscle and obtained a variant of reduced virulence for monkeys. Sabin, Hennessen, and Winsser (2) have obtained variants of Mahoney (type I), Y-SK (type II), and Lcon (type III), which are also relatively avirulent in monkeys, through passage at 1-day intervals with large inocula in tube cultures of cynomolgus monkey kidney cells. Li, Schaeffer, and Nelson (3) have combined passages in vitro with passages in vivo to obtain variants of Mahoney and Leon which show various patterns of virulence for mice and monkeys. Melnick (4) has also reported attenuation of polio viruses through serial passages of high concentrations of virus in tissue culture. Dulbecco and Vogt (5) have obtained an r (rapid) mutant of Brunhilde through serial rapid passage on monolayer cultures of cynomolgus monkey kidney cells. Slow Mahoney (6), a genetic variant of Mahoney that produces relatively tiny plaques on monolayers of monkey kidney cells, was isolated after propagation of the parental Mahoney on HeLa cells. In the work reported here (7), cold-adapted genetic variants of the polio virus strains Akron (type I), Brooks (type II), and Mabie (type III) have been obtained through passage at 30°C on monkey kidney cells.

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