animals of Exp. 1 and 4.

In none of these controls were bodies found which were similar to the pre-erythrocytic stages observed in the infected animals. The control material did demonstrate the need for caution in interpreting the data, for in these animals there were some forms such as degenerating liver cells and thrombi which might have been mistaken for parasites.

Discussion. The observations reported above suggest that the morphological characteristics of the pre-erythrocytic stages of *P. cynomolgi* are remarkably like those of the pre-erythrocytic stages of avian malaria. Certainly the tissue stages of *P. cynomolgi* are more similar to those of *P. gallinaceum* than of *Hepatocystes* (*Plasmodium*) *kochi*.[14] The exoerythrocytic stages of *Hepatocystes* resemble those stages observed in *Leucocytozoan* infections of ducks[15] while the *P. gallinaceum* and *P. cynomolgi* stages appear much like those of *Hemoproteus*. An obvious difference exists in that hepatic cells are not invaded by avian malaria and *Hemoproteus* whereas they may be in simian malaria, *Hepatocystes* and *Leucocytozoan*. Since *P. mexicanum* in the lizard[16] has both elongatum and gallinaceum type exoerythrocytic stages, it is to be expected that other species of malaria could have diverse host cell developmental potentialities.

Summary. Pre-erythrocytic stages of *P. cynomolgi* have been observed in Kupffer cells, hepatic cells, and mononuclear phagocytic cells of the liver, as well as in large mononuclear phagocytic cells of the spleen, probably reticular in nature.

[14] Garnham, P. C. C., *Tr. Roy. Soc. Trop. Med. and Hyg.*, 1948, **41**, 601.

[15] Huff, C. G., *J. Infect. Dis.*, 1942, **71**, 18.

[16] Thompson, P. E., and Huff, C. G., *J. Infect. Dis.*, 1944, **74**, 48.

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Determination of Carbon 14 in Fatty Acids by Direct Mount Technic.*

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The measurement of C^{14} in fatty acid fractions prepared from animals into which a C^{14}-labeled fatty acid has been introduced is beset with considerable difficulty. A sample of high specific activity is not often obtained because the administered radioactive fats are diluted by a factor of at least 1000 when mixed with the body pool of fatty acids. The activity per unit of mass is further reduced by a factor of 17 when the conventional $BaCO_3$ technics are used. The first dilution—that due to mixing with the body pool of fatty acids—obviously cannot be avoided. In order, however, to circumvent a further reduction of the activity, it seemed desirable to investigate direct mounting of fat samples.

When samples of C^{14}-containing fatty acids were mounted directly on bare aluminum discs, reproducible values for counting rates were not obtained because the material collected on the surface of the discs in the form of globules. Reproducible results were obtained by employing a cover of lens paper which brought about an even distribution of the fatty acids on the disc. The degree of reproducibility that can be attained by mounting radioactive fatty acids on lens paper-covered aluminum discs is shown in Table I which records the results of quadruplicate determinations for each of 3 different fatty acid samples. The deviations from the averages did not exceed 5%. The degree of reproducibility which can be at-

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TABLE I.
Reproducibility of C^{14} Counting When Fat Was Mounted on Lens Paper-covered Aluminum Discs.
(In each experiment 4 one-cc aliquots of a solution of C^{14}-labeled palmitic acid in corn oil were separately mounted.)

Fatty acid sample	Counts per minute					% deviation from avg activity
	Per mount				Avg	
1	2780	2790	2780	2750	2775	0.5
2	3720	3610	3610	3810	3690	2.1
3	4430	4500	4380	4460	4440	0.8

tained in the counting of C^{14} by this method, when employed by different individuals was also investigated and it was found that the variation in the counts obtained by 3 individuals did not exceed 5%.

Although the method described above was designed primarily to avoid the dilution of activity inherent in the preparation of $BaCO_3$ mounts, its simplicity also permits a considerable saving of time. Fifty or more samples can be readily mounted by a single individual in a period of 8 hours.

A comparison of C^{14} activity as measured by direct mount technic with that measured as $BaCO_3$. It is frequently necessary to compare the specific activity of a fatty acid fraction with that of a non-lipid fraction or that of expired CO_2. Since at present the activity in the two latter materials is usually determined after conversion to $BaCO_3$, it became necessary to relate the activity of fat as measured by the direct mounting technic described below to its activity when measured in the form of $BaCO_3$.

Corn oil fatty acids were mixed with a sample of C^{14}-labeled palmitic acid and the mixture dissolved in petroleum ether. Suitable dilutions were then prepared so that each one-cc aliquot contained from 2.5 to 35 mg of fatty acids. Each sample was directly mounted and its activity determined. Duplicate samples were oxidized to CO_2 and converted to $BaCO_3$ and the activity of the $BaCO_3$ determined. All $BaCO_3$ counts were corrected to a standard mass of 40 mg according to the method of Henriques *et al.*[1]

[1] Henriques, F. C., Jr., Kistiakowsky, G. B., Margnetti, C., and Schneider, W. G., *Ind. Eng. Chem. Anal. Ed.*, 1946, **18**, 349.

The values for the ratio:

$$\frac{\text{Corrected counts from } BaCO_3}{\text{Counts from directly mounted fatty acids}}$$

were determined for many samples. A plot of these values as ordinates against mg of fatty acids used in the direct mount as abscissa is shown in Fig. 1. From this empirical curve, the factor for converting counts obtained from a direct mount to the activity that would have been obtained had the C^{14} of the fatty acids been measured after conversion to $BaCO_3$ can be read.

In order to test the reliability of the conversion factors, 5 samples of tissue fatty acids isolated from rats that had received C^{14}-labeled palmitic acid were mounted directly in duplicate and the activities determined. The values obtained were then converted to the $BaCO_3$ basis by using the factors in Fig. 1. Aliquots of these same 5 samples were then oxidized to CO_2 and their activities determined in the form of $BaCO_3$. The results are shown in Table II.

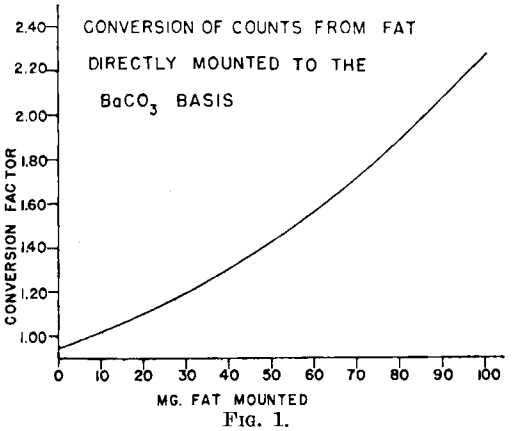

FIG. 1.
Factors for conversion of activity as measured by direct mounting technic to activity on a $BaCO_3$ basis.

TABLE II.
Reliability of Empirical Conversion from Counts per Minute per Mg Tissue Fatty Acids to Counts per Minute per Mg $BaCO_3$.

1	2	3
	Counts per Minute per Mg $BaCO_3$.	
Mg fatty acids	Calc. from direct mount by use of factors in Fig. 1	Found by oxidation of fatty acids to $BaCO_3$
5.7	10.8	10.3
5.7	10.6	10.2
12.9	10.8	10.3
13.3	10.4	10.1
17.9	11.0	11.1
18.1	10.6	10.3
22.5	10.9	10.3
22.9	11.0	10.3
28.1	10.9	10.5
27.9	11.1	10.1

The values obtained by this conversion (col. 2) are in good agreement with those obtained by direct oxidation of the fatty acids to $BaCO_3$ (col. 3). This agreement, therefore, justifies the use of the conversion factors and the determination of the radioactivity in fatty acids by the direct mount technic, a procedure that yields the highest activity per unit of mass.

Experimental. Direct mounting of fatty acids on lens paper-covered aluminum discs. An aluminum disc 1.75 inches in diameter, lined with a piece of lens paper of the same diameter, was weighed and then placed approximately 6 inches below an infra-red lamp. An aliquot of a fatty acid solution (usually one cc) containing the C^{14}-labeled fatty acid was added dropwise to the warmed lens paper at a rate that kept the surface constantly and uniformly wet but prevented the fat solution from creeping beyond the edge of the disc. The disc and its contents were then reweighed. It was found that, by this method, about 40 mg could be safely mounted on a single disc without encountering loss from creeping. If a concentrated solution of fatty acids in ether is evaporated by using an air stream instead of an infra-red lamp, however, as much as 150 mg of fatty acids can be mounted on a disc. The activity of the mounted material was measured by a thin mica window Geiger tube.

Satisfactory results with lens paper which spreads the solution evenly have been reported by Fager.[2] His procedure, however, differs from that described above.

Wet combustion of fatty acids. The apparatus (Fig. 2) used for wet oxidation is a modification of that described by Skipper *et al.*[3] Potassium iodate was omitted from the combustion fluid. Concentrated H_2SO_4 or the combustion fluid was used as a joint lubricant. The tapered joints of the standard glass-stoppered 125 cc Erlenmeyer flasks occupy a portion of the 19/38 joints (Fig. 2). The samples were pipetted into the flasks and the solvent evaporated on the steam bath, the last traces being blown out with nitrogen. After the apparatus was assembled, the vacuum line was opened and CO_2-free air drawn through at a rate sufficient to provide the necessary dispersion in the NaOH tower. A measured amount of the combustion fluid was then added through the funnel, and the flask heated gently until fumes first appeared. This temperature, or a slightly lower one, was maintained for 5 minutes. Care was taken to avoid the excessive production of SO_3 fumes.

[2] Fager, E. W., Reported in Symposium on the Use of Isotopes in Biological Research, University of Chicago, March, 1947.

[3] Skipper, H. E., Bryan, C. E., White, L., Jr., and Hutchinson, O. S., *J. Biol. Chem.*, 1948, **173**, 371.

The flame was removed and CO_2-free air passed through the apparatus for an additional 10 minutes. The $Na_2C^{14}O_3$-NaOH was next forced into a volumetric flask by means of air pressure. Aliquots were taken for the precipitation and mounting of $BaCO_3$ and for the determination of CO_2.

The completeness of oxidation under the conditions described above was tested for the following compounds: palmitic acid, cholesterol, glucose, and corn oil. Six mg of the first 3 compounds were used whereas corn oil was tested over a range of 5 to 30 mg. In all cases, the recovery of CO_2 was from 95 to 100% of the theoretical.

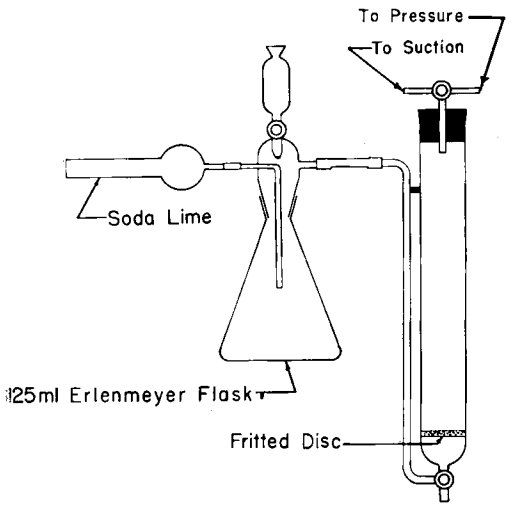

FIG. 2.
Apparatus used for oxidation of organic compounds.

Determination of CO_2. An excess of a saturated $BaCl_2$ solution was added to aliquots of the $Na_2C^{14}O_3$ solution. This solution was then titrated with dilute HCl to the phenolphthalein end point. Brom cresol green was then added, and the solution titrated with standard 0.1 N HCl to the new end point. The amount of $BaCO_3$ precipitated was calculated from the titration difference which had been corrected for a blank titration value.

Preparation of the $BaCO_3$ mount.† An excess of a saturated $BaCl_2$ solution was added to an aliquot of the $Na_2C^{14}O_3$ solution. Suction was applied to the filtration apparatus (Fig. 3) and the suspension of $BaCO_3$ poured into the glass cylinder. The suspension was allowed to filter completely and the precipitate, while still moist, was washed first with water and next with acetone. Suction was maintained constantly. After removal of the glass cylinder, an infra-red lamp was placed one inch above the surface of the mount and hot air was pulled through the mount for several minutes. Suction was discontinued at this point. The paper and $BaCO_3$ were removed as a flat mount. (There should be little or no curvature.) For determination of its radioactivity, the mount was held flat by means of the carrier shown in Fig. 4. No loss of activity has been observed in samples kept in covered lucite trays for as long as 11 weeks.

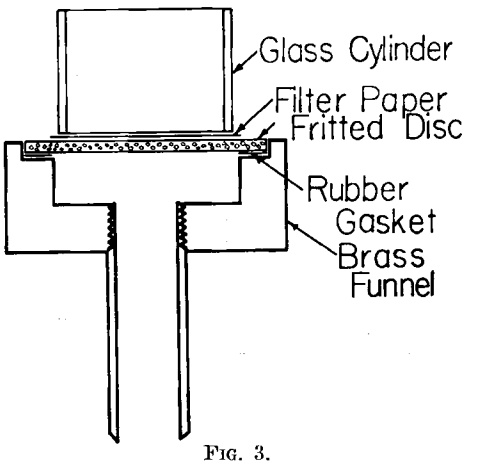

FIG. 3.
Filtration apparatus for preparation of $BaCO_3$ mounts. The filter paper used is Whatman No. 42. The glass cylinder is held firmly in place by means of two elastic bands attached to glass ears on the sides of the cylinder and to metal screws on the sides of the brass funnel.

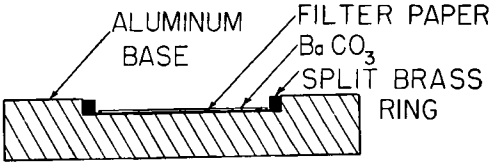

FIG. 4.
Holder for keeping $BaCO_3$ filter paper mounts flat during counting.

† Similar mounting technics have been described for benzidine sulfate[1] and P^{32} in plant material.[4]

[4] MacKenzie, A. J., and Dean, L. A., *Anal. Chem.*, 1948, **20**, 559.

TABLE III.
Reproducibility of C^{14} Determination When $BaC^{14}O_3$ Was Mounted on Filter Paper.

Sample	$BaCO_3$ mounted, mg	Counts per min.		Specific activity of $BaCO_3$, counts per min. per mg	% difference between duplicate specific activities*
		Observed	Corrected for mass		
A	57.3	725	860	15.0	4.5
A	57.8	765	910	15.7	
B	76.1	1370	1920	25.2	1.6
B	74.5	1330	1850	24.8	
C	54.5	925	1060	19.5	4.5
C	53.4	955	1090	20.4	
D	43.5	329	342	7.9	3.9
D	44.1	323	336	7.6	
E	43.4	143	149	3.4	2.9
E	43.0	148	152	3.5	

* These values include errors of titration and counting.

After its radioactivity had been determined, the $BaCO_3$ mount was transferred to an Erlenmeyer flask and an excess of standard 0.1 N HCl added. After the reaction was complete, the excess acid was titrated to the brom cresol green end point with standard alkali. The weight of the $BaCO_3$ was calculated from the equivalents of acid consumed.

For permanent mounts, or as an alternative to the $BaCO_3$ titration procedure, the filter paper can be weighed before and after the $BaCO_3$ is mounted. For samples of small weight, the filter paper should be dried at 120° before each weighing.

In order to test the reliability of the mounting of $BaCO_3$ on filter paper, the following experiments were carried out. Five $Na_2C^{14}O_3$ solutions (A to E, Table III) of varying radioactivity were used. From each solution, 2 mounts were prepared on filter paper (as described above) and their C^{14} activity determined. The weight of the $BaCO_3$ of each mount was calculated from titration values. The 2 values found for the specific activity of each sample (Table III) were in good agreement, no pair differing by more than 5%.

The data presented here apply only to the particular geometry of the counting device employed in this laboratory and hence cannot be applied to cases where the counting arrangements differ.

Summary. It is demonstrated in the present investigation that the C^{14} activity of a fatty acid sample can be readily determined by a direct procedure that avoids the dilution of activity and laboriousness associated with the preparation of $BaCO_3$ mounts. The simplicity of this new procedure permits a single operator to mount as many as 50 samples in 8 hours with an error of reproducibility not in excess of 5%. By means of an empirically constructed curve, the observed activities can be converted to a $BaCO_3$ basis.