groups of eggs on the absolute time scale, the normal eggs should be dissected at stage 10, and cultivated until the hybrid eggs of the same clutch are ready, and then measured simultaneously. One experiment has been carried out with region 1 as a test. This region of the normal egg was isolated first and cultivated under the same condition as the hybrid eggs. After the hybrid eggs reached stage 10, the corresponding region of the hybrid gastrula was dissected out. When the healing of the hybrid explant was complete, both the normal and the hybrid explants were measured simultaneously. The rates of the normal and the hybrid explants were, respectively, 4.17 and 1.60×10^{-4} $\mu l/\mu g/hr$ with respect to dry weight. The normal explant has a respiratory activity 2.6 times greater than the hybrid one.

The results of the experiments suggest that the effect of the *R. sylvatica* genome on respiration is widespread. There is no localized effect; that is, all cells are apparently affected similarly, since the general respiratory pattern of the hybrid gastrula remains the same as that of the normal gastrula, the only difference between these two kinds of eggs lying in the magnitude of the respiratory activity. These results are in agreement with the conclusion of Barth (*4*) and the results from experimental embryological studies of Moore (*1, 2*).

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Liquid Scintillation Counting of Tritium-labeled Water and Organic Compounds[1]

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The liquid solution scintillator is assuming an increasingly important role for homogeneous counting of C^{14} compounds. With such compounds as steroids, fatty acids, etc., which have little quenching action on the scintillation system, relatively large samples may be counted with high experimentally realizable efficiency (40–60%). The high efficiency, ease of operation, and the large range in sample size provide ample reason for establishing liquid scintillation counting as the method of choice in many biological experiments utilizing C^{14}. Solutions that have been employed for scintillation counting include *p*-terphenyl in toluene (*1*), 2,5-diphenyloxazole in toluene (*2*), and *p*-terphenyl in various mixtures of dioxane and water (*3*).

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Instrumentation problems associated with liquid solution scintillation counting of low energy β-particles have been partly resolved by coincidence circuitry (*1, 2*) and refrigeration of photomultiplier tubes in order to lower the portion of the background counting rate that is due to dark current. The upper level discriminator operating in anticoincidence (*2*) has proved useful for rejecting high amplitude background pulses and improving the counting rate ratio of sample to background.

The considerably lower energy β-spectrum of H^3 relative to C^{14} indicates the probability of a correspondingly greater difficulty in realizing satisfactory counting conditions for tritium. The observation that tritium-labeled hexestrol (*4*) could be counted with low efficiency in the C^{14} scintillation system (*2*) suggested further investigation of this type of tritium counting.

Using the fast coincidence instrument designed for C^{14} scintillation counting, various tritium-labeled steroids and fatty acids were counted, and the counting efficiency was estimated by assay of the water obtained on combustion. A vibrating reed electrometer was used to compare the water with a standard tritium water sample (*5*). In addition, it was found possible to count tritium water directly with about the same sensitivity as with the electrometer, and with considerably greater rapidity and convenience.

2,5-Diphenyloxazole was employed as the solute with toluene, dioxane, and xylene as the major solvents. The standard practice was to use 100 mg solute in a total volume of 30 ml solution. Miscibility of tritium water with toluene or xylene was achieved by addition of absolute ethanol.

Contamination difficulties were minimized by use of Pyrex weighing bottles (45 mm in diam by 65 mm) as sample containers. These can be swiftly inserted into, and removed from, the area between the photomultiplier cathodes, thus permitting samples to be changed without warming of the tubes. The sample holders also may be easily cleaned or discarded when significantly contaminated.

Coincidence counting efficiencies reported here were accompanied by backgrounds of 100–200 cpm. Efficiencies from either one of the single tubes were 2–4 times higher than those from the two tubes operated in coincidence, but the backgrounds were of the order of 10,000–100,000 cpm.

Results obtained with a few sterols dissolved in toluene are given in Table 1. Samples ranging in size from fractions of a milligram to a gram may be counted with the same efficiency, the upper limit of sample size being conditioned only by the solubility of the compound. Although certain organic compounds exhibit a quenching effect, none of those reported in Table 1 showed perceptible quenching in the concentrations reported.

The counting data obtained for tritium water are presented in Fig. 1 and Table 2. The highest efficiency was realized with 0.01 ml water, 0.19 ml absolute

TABLE 1

SCINTILLATION COUNTING DATA FOR TRITIUM-LABELED STEROLS IN TOLUENE SOLUTION

Tritium-labeled compound	Mg assayed	Mg inert form added	Cpm	Cpm/mg of labeled compound	Ion chamber results (μc/mg)*	Efficiency of scintillation counting (%)
Cholesterol-6,25-T_2	6.7	0	29,000	4,329	0.0315	6.41
	0.266	13.03	1,270	4,775		
	0.1067	999.9	463	4,340		
Cholestanol-5,6-T_2	0.064	31.93	4,042	63,100	0.442	6.44
Sitosterol-T	5.6	0	52,294	9,695	0.068	6.48
	0.0474	23.65	454	9,580		
	0.0167	167	168	10,060		
Δ^7-Cholestenol-5,6-T_2 (Lathosterol)	0.5	5.0	45,550	91,100	—	—

* Each sterol was diluted with the inert form to a specific activity of 0.02–0.08 μc/mg, burned in a combustion tube, and the resultant water assayed in a vibrating reed electrometer.

ethanol, and 29.8 ml toluene. The decrease in efficiency with increasing amounts of water is due to the dilution of the effective solvent; the decrease at small concentrations is apparently due to adsorption of the tritium water on the glass. The latter effect has not been observed in tritium sterol or in C^{14} counting. A convenient procedure for preparing tritium water samples for counting is to add absolute alcohol to the sample to give either 90% or 95% alcohol. An aliquot of the dilute alcohol is then added to a solution of 100 mg of 2,5-diphenyloxazole in sufficient toluene or xylene to give a total volume of 30 ml.

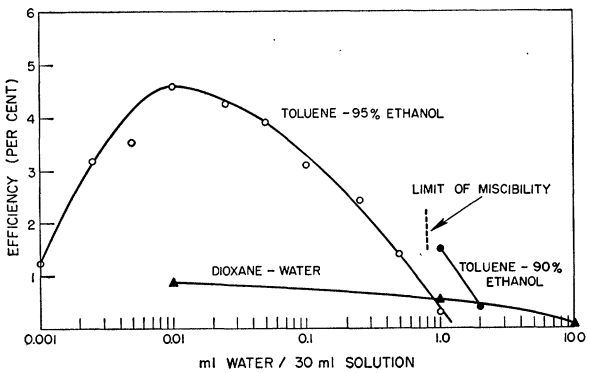

FIG. 1. Efficiency of scintillation counting of tritium water in toluene-alcohol and in dioxane.

When maximum sensitivity is desired, and large amounts of sample are available, the optimal procedure at present consists of using a mixture of 20 ml xylene, 100 mg 2,5-diphenyloxazole, and 10 ml 90% alcohol made from the tritium water sample and absolute alcohol. This procedure gave a counting efficiency of 2.0%, using xylene as the solvent, and 1.5% using toluene.

The principal advantage of using dioxane as solvent is that it is not necessary to add ethanol to produce miscibility of the water sample. However, the efficiency of dioxane as a scintillation solvent is sufficiently inferior to toluene and xylene that the latter solvents provide maximum sensitivity, although dioxane permits the counting of much larger samples.

In the counting range studied all three solvents gave sensitivities comparable to that obtained with a vibrating reed electrometer, as the lower efficiency is balanced by the much greater sample measurable.

Assuming the lower level of significance for the sample counting rate to be 75 cpm above background, the lower limit of detectable specific activity would be

$$\mu c/ml_{11m} = \frac{75}{2.22\times10^6}\,\frac{100}{VE} = \frac{3.4\times10^{-3}}{VE},$$

where V is the volume of the HTO sample in ml and E is the percentage counting efficiency. In the experiment involving 20 ml xylene, 10 ml 90% ethanol, and 100 mg 2,5-diphenyloxazole, this limiting value is 1.7×10^{-3} μc/ml.

The general nature of the dilution effect and the specificity of adsorption problems to tritium water counting were demonstrated by counting C^{14} samples in tritium-free solutions. Efficiencies shown in Table 2 are based on benzoic acid prepared from National Bureau of Standards C^{14}-containing sodium carbonate.

TABLE 2

THE EFFICIENCY OF SCINTILLATION COUNTING OF TRITIUM WATER AND C^{14} BENZOIC ACID IN VARIOUS SOLVENT MIXTURES

Toluene (ml)	30	30	29.9	29.8	29.5	29	28	25	20	10	20	20*	10	30†	29.9‡	29‡	20‡
Absolute ethanol (ml)	0.019	0.0475	0.095	0.19	0.475	0.95	1.9	4.75	9.5	19	9	9	18	20	—	—	—
Water (ml)	0.001	0.0025	0.005	0.01	0.025	0.05	0.1	0.25	0.5	1.0	1.0	1.0	2.0	2.0	0.1	1.0	10
H^3 efficiency (%)	1.3	3.2	3.6	4.6	4.5	4.2	3.9	2.4	1.4	0.3	1.5	2.0	0.4	0.8	0.9	0.6	0.04
C^{14} efficiency (%)	55	54	54	53	52	50	48	39	32	14	31	37	13	23	30	23	24

* Xylene.
† 52 ml total volume and 200 mg 2,5-diphenyloxazole.
‡ Dioxane.

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Concerning the Site of Nitrogen Absorption in Rats Fed Autoclaved or Raw Soybean Oil Meal[1]

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Carroll, Hensley, and Graham (*1*) have concluded that much of the nitrogen absorption in rats fed raw soybean oil meal must take place in the cecum. This conclusion was reached from data showing that the apparent digestibility of raw soybean nitrogen in the terminal 20% of the small intestine was 32.65%, whereas in the feces the value was 76.96%. Values reported for apparent digestibility of heated soybean nitrogen were 78.66% and 81.78%, respectively. This observation presented a notable advance in explaining the lower nutritive value of raw soybeans compared with autoclaved soybeans. It therefore seemed advisable to repeat this work in order to determine the validity of the observations made.

The Cr_2O_3 index procedure was employed in a manner similar to that of Carroll *et al.* (*1*), with the following pertinent notations. The autoclaved and raw soybean rations contained 2.1% total nitrogen and were compounded as in previous studies (*2*). Rats of the Sprague-Dawley strain were fed the respective ration for a period of 4 days before being sacrificed.

TABLE 1

Apparent Digestibility of Soybean Nitrogen in the Terminal 20% of the Small Intestine

Series	No. of rats	Av wt (g)	Apparent digestibility ± SE (range) Soybean oil meal		t value
			Autoclaved (%)	Raw (%)	
1	20	126	73.35 ± 1.92 (52.7–84.8)		1.968*
	20	120		66.90 ± 2.66 (43.3–82.8)	
2	21	189	78.52 ± 0.93 (71.7–84.5)		1.644*
	18	186		75.17 ± 1.82 (59.1–86.5)	

* Not significantly different, t value according to Snedecor (*5*).

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The determination of Cr_2O_3 was carried out by the method of Schürch *et al.* (*3*), except that the dichromate color was read at 375 mμ, as suggested by Dansky and Hill (*4*).

The data accumulated in our experiments indicate that the apparent digestibility of raw soybean nitrogen was not significantly different from the apparent digestibility of autoclaved soybean nitrogen when determined by the Cr_2O_3 index method in the terminal 20% of the small intestine of the rat. The results are presented in Table 1.

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A Test Utilizing the *in Vitro* Clearing of Milk to Determine the Presence of Lipid Clearing Factor in Plasma

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Recent studies indicate that certain physically distinct lipoprotein particles suspended in the blood may be etiologically significant in atherosclerosis (*1, 2*). Following an earlier observation that heparin administered intravenously decreased the turbidity of plasma during alimentary lipemia (*3*), a number of workers have been investigating the possibility that the heparin clearing phenomenon may be of significance in lipid metabolism, particularly in relation to the etiology of atherosclerosis. The clearing effect has been found to occur *in vitro* when lipemic plasma is incubated with plasma withdrawn from donor animals soon after the intravenous administration of heparin (*4*). Subsequent work suggests that a soluble tissue substance, in the presence of heparin, catalyzes the conversion of a precursor present in plasma to a lipid clearing factor. The latter apparently effects a redistribution of plasma lipids in such a way that turbidity is decreased (*5*).

We have been testing for the presence of clearing factor precursor in the plasma of normal and abnormal subjects in connection with our work on postprandial serum turbidity in atherosclerotic patients (*6*). In the course of our study we have investigated the possibility of substituting for lipemic plasma a more easily procured and more readily standardized substance as testing material in the assay of lipid clearing factor. We have found that milk fulfills these requirements reasonably well.

[1] Fellow of the Heart Association of Southeastern Pennsylvania.