TABLE 1

	R. hawaiiensis	*R. norvegicus*
Number of animals	20	10
Mean weight of test animals	48.0 ± 1.8 g	186.7 ± 14.7 g
Number surviving 34 days of alternate feeding	15	0
Minimum day of death	7 days	4 days
Maximum day of death	34 days	11 days

regimen was continued until the animal died or 34 days of alternate exposure to warfarin-treated oats and plain rolled oats had occurred.

The results obtained are presented in Table 1.

The alternate feeding of warfarin oats and plain oats to *Rattus norvegicus* gave a kill identical to the results obtained by Doty (*1*) with continuous feeding of the same poison bait mixture. All individuals died in 11 days or less and showed evidence of internal hemorrhages on autopsy. On the other hand, with *R. hawaiiensis*, 75% of the test individuals survived more than 34 days of this alternate feeding of warfarin-poisoned oats and plain rolled oats. This period is double the 17-day period of feeding recommended by Doty as a practical time limit for field poisoning. The average consumption of poison bait during the 34-day period was approximately twice the average amount that had been previously found to kill this species when consumed on a daily basis (*2*). These data indicate that unsatisfactory results will be obtained in the case of *R. hawaiiensis* unless the present warfarin bait is consumed at a frequency greater than every other day. Very little is known of the feeding habits of *R. hawaiiensis* in its native habitat, but the results obtained in the field test (*3*), in the light of the present data, would seem to indicate that in spite of a heavy consumption of the bait in the field, individual *R. hawaiiensis* probably did not consume the bait consistently on a daily basis, and hence survived the poisonous effects of the warfarin.

The fifteen *Rattus hawaiiensis* individuals that survived 34 days of alternate feeding and poisoning were placed on a continuous diet of warfarin oats bait on the 35th day. They all died as a result of warfarin poisoning, and the mean day of death was 13.5 ± 0.9 days after the beginning of this continuous period of warfarin feeding.

TABLE 2

	R. hawaiiensis Present experiment	*R. hawaiiensis* Previous experiment (*2*)
No. of individuals	15	42
Previous exposure to warfarin	Every other day for 34 days	None
Total body weight	959.3 g	2313.8 g
Mean body weight	49.6 ± 1.7 g*	55.1 ± 1.2 g
Total warfarin oats consumed	373.5 g	899.0 g
Total warfarin oats % of total body weight	38.9 ± 2.0%	38.9 ± 1.3%
Mean day of death	13.5 ± 0.9	7.9 ± 0.5

* The lesser weight of the animals of this series is due to an overall weight loss during the 34-day exposure period. The initial weight of this group was 52.0 ± 2.5 g.

The results obtained in this test are compared in Table 2 with the results obtained in the test already reported (*2*) on animals which had no previous exposure to warfarin.

Although the quantity of warfarin oats bait consumed in terms of percentage of body weight is the same in both series (38.9%), the mean number of days elapsing before death is significantly greater in the series previously exposed to warfarin bait on an alternate day basis. The physiological basis of this finding has not been determined but it is a possibility that the test animals had no avidity for food as a result of the probable sublethal poisoning over the 34-day period and hence took a longer time to consume the quantity of warfarin oats necessary to kill.

References

1. Doty, R. E. *Hawaiian Planters' Record*, **54**, (1), 1 (1950).
2. Bonnet, D. D., Mau, E. S. C., and Gross, B. *Public Health Repts. (U.S.)*, **66**, 1734 (1951).
3. Gross, B., Baker, R. H., and Bonnet, D. D. *Ibid.*, 1727.
4. Eskey, C. R. *Public Health Bull.*, No. 213, 1 (1934).

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Synthesis of DDT Labeled with Carbon-14 in the Tertiary Position

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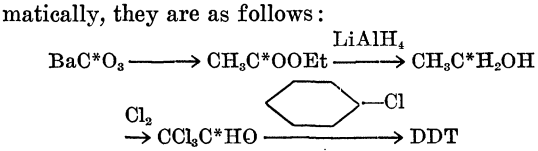
A method has been developed for the synthesis of radioactive Dichlorodiphenyltrichloroethane (DDT) in good yield in which the tertiary carbon is labeled.

Fields *et al.* (*1*) have developed a satisfactory procedure for incorporating C^{14} in the benzene ring. This procedure presumably was employed in a previously reported labeling of DDT with C^{14} (*2*). However, the complete synthesis of DDT by this procedure would involve at least seven major steps, with the consequent tendency towards low over-all yields. In the authors' procedure, only four major steps are necessary. Schematically, they are as follows:



The synthesis of carboxy-labeled ethyl acetate was carried out by special adaptations of the methods of Sakami *et al.* (*3*), Van Bruggen *et al.* (*4*), and Ropp (*5*). The ester was reduced to ethyl alcohol by a modification of the method of Cox and Turner (*6*). Chlorination was carried out in a specially designed apparatus,

and the subsequent condensation of chloral with chlorobenzene followed the work of Mosher *et al.* (*7*).

Starting with 50 mM of barium carbonate containing 20 mc of activity, 15 g of crude DDT (42% yield based on EtOH) was obtained. Two crystallizations from ethyl alcohol yielded 6.11 g of *p,p'* DDT (17% yield) having a melting point of 107–107.5° C. The specific activity of the product was approximately ½ mc/g.

The residue is currently being treated to recover additional amounts of the *p,p'*-isomer as well as the *o,p*-isomer.

It is planned to submit a detailed report of this work elsewhere.

References

1. FIELDS, M., LEAFFER, M. A., and ROHAN, J. *Science,* **109**, 35 (1949).
2. Tracerlog No. 31, p. 10 (1950), Tracerlab, Inc.
3. SAKAMI, W., EVANS, W. E., and GURIN, S. *J. Am. Chem. Soc.,* **69**, 1110 (1947).
4. VAN BRUGGEN, J. T., CLAYCOMB, C. K., and HUTCHENS, T. T. *Nucleonics,* **7**, 45 (1950).
5. ROPP, G. A. *J. Am. Chem. Soc.,* **72**, 2299 (1950).
6. COX, J. D., and TURNER, H. S. *J. Chem. Soc.,* 3176 (1950).
7. MOSHER, H. S., *et al. Ind. Eng. Chem.,* **38**, 916 (1946).

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Protamine, Chylomicrons, and Clot Formation[1]

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The main intestinal lymph channels of 14 rats fed 1 ml of corn oil were cannulated by the technique of Bollman *et al.* (*1*). Addition of 0.2 mg of protamine (clupein, Vitrum, Stockholm) to 1 ml of chyle caused immediate flocculation of the chylomicrons. The floccules rose to the surface during the following 30 min leaving a clear subnatant fluid. Five samples of chyle were adjusted to pH 7.4 with *N* HCl and 0.1 ml aliquot parts from each sample were mixed with quantities of protamine ranging in weight from 2.5 to 20 μg in multiples of 2.5 μg. The minimum quantity of protamine causing flocculation varied from 0.05 to 0.20 mg per ml of chyle. The pH of aliquots of normal chyle (7 samples) and chyle containing flocculated chylomicrons (5 samples) were gradually changed from 7.4 to 2 or 12, and the isoelectric point of normal chylomicrons in intestinal lymph was determined by the technique of Ludlum *et al.* (*2*). This was found to lie between pH 4.6 and 4.9. Lymph chylomicrons in the human subject are apparently stabilized with albumin (*3*) (isoelectric point pH 4.7), and it is clear that the same is true of the rat. The protamine-flocculated chylomicrons dispersed to form a stable emulsion over the pH range less than 4.7 and flocculated again if the pH was readjusted to a value greater than 4.7. Evidently protamine is adsorbed to the interface of lymph chylomicrons in the interisoelectric region of albumin and protamine (pH 4.7–12) causing loss of surface potential followed by flocculation. The reaction appears to be similar to that discussed by Elkes *et al.* (*4*) in the course of examination of effects of proteins on detergent-stabilized emulsions.

Protamine was added to 7 chyle samples (1 mg/ml) and allowed to stand for 30 min. The samples were then centrifuged at 3000 rpm for 2 min when a crystal-clear subnatant fluid was obtained. It is possible that lymph clarified in this manner contains all the non-chylomicron lipid and that the procedure can be used to facilitate analysis of the composition of lymph chylomicrons, or the fluid in which they are suspended. The mean total fatty acid content determined by a modified Schmidt-Nielsen technique (*5, 6*) of the whole chyle samples and clear subnatants was, respectively, 3000 (range 5000–1500) mg%, 34 (60–12) mg%. These observations suggest that the fat in intestinal lymph is almost entirely in chylomicron form during fat absorption, but it remains to be shown that only the chylomicrons are separated from lymph by this treatment. However, no differences in the distribution of proteins in thrombin-treated chyle before and after clarification by protamine were detected (*7*) by the Tiselius electrophoretic technique.

The samples of normal chyle which were collected during the present investigation usually slowly clotted on standing. The clots did not retract and they bound large quantities of lymph which were difficult to expel by compaction. The chylomicrons remained suspended in the lymph fluid recovered by compaction and imparted to it the characteristic milkiness of freshly drawn chyle. In several of the chyle samples treated with protamine, formation of a light clot and flocculation of the chylomicrons occurred within several seconds of treatment. The clot bound chylomicrons so that when it was lifted out of the lymph, the chylomicrons were lifted out with it leaving partially or completely clarified lymph behind. It did not bind lymph fluid and, in this respect, on removal from the lymph it resembled a compacted clot from normal lymph. The reaction was evidently associated with surface changes on the chylomicrons, and it was not a clotting reaction in the usual sense.

Blood was drawn 3 hr after feeding 8 rats 4 ml of olive oil/kg body wt. The effect of protamine on the serum chylomicrons visible in the darkfield microscope (1200 ×) was ascertained. In all 8 samples the chylomicrons were flocculated into immobile clumps by 2 to 3 mg of protamine/ml of serum; 1 mg/ml had little or no visible effect. Thus the minimum dose of protamine causing flocculation of chylomicrons in serum is considerably higher than that required to flocculate chylomicrons in intestinal lymph. Addition of 3.3 ml of a protamine sample/ml of serum induced changes in the electrophoretic pattern of serum owing to association of albumin with protamine (*8*), and it may well be that the larger dose required to flocculate the serum chylomicrons is due to competition between

[1] Many samples of chyle used in this investigation were supplied by B. Borgström and R. Blomstrand.