an activator of plasminogen is possible by comparing effects on normal bovine fibrin (which contains plasminogen) and heated bovine fibrin (with no plasminogen) (10). Extracts from the rat and rabbit uterus digested normal bovine fibrin whereas no digestion was obtained on heated fibrin.

The "enzyme" of Page *et al.* increased in concentration during estrogen withdrawal whereas castration caused a decrease. Contrary to these findings we found no decrease in tissue activator concentration after castration of rats. The discrepancy between our results on rats and those of Page *et al.* on rabbits could be caused by differences between the animal species.

Summary. 1. Concentration of tissue activator of plasminogen in the rat uterus has been estimated in different stages of the estrous cycle. 2. An increase in concentration was recorded during late estrus, whereas castration had no influence upon the concentration. 3. These results are in accordance with those found for human endometrium and myometrium.

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Metabolism of Esters by *Streptomyces nitrificans.** (23058)

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Considerable attention has been devoted to the inhibitive effects of physostigmine (eserine), prostigmine, miotine and other carbamates on acetylcholine esterase and cholinesterase(1,2), which are representative of the so-called "azolesterases"(3). Much less work has been done on effects of urethans on ordinary esterases, *i.e.* aliesterases, and on the enzymatic hydrolysis of carbamic acid esters(1,4). For example, eserine but not urethan inhibited a mycobacterial esterase that hydrolyzed tributyrin, ethyl and amyl butyrates, butyl propionate and benzoate, and ethyl valerate(5). In another study, 9 different urethans were attacked by a pig liver esterase extremely slowly if at all, but interfered with hydrolysis of methyl butyrate and tributyrin(6).

This difference in susceptibility of carbamic acid esters to pig liver esterase motivated the present investigations on metabolism of esters by *Streptomyces nitrificans,* an actinomycete which hydrolyzes(7,8), respires (9,10), nitrifies(8,9,11) and grows(7,10,12) on urethan and other carbamates. In an extensive survey of soil microflora, *S. nitrificans* was the only organism capable of developing in a mineral solution with ethyl carbamate as the source of available nitrogen, carbon, and energy(7). This unique culture was therefore assumed *a priori* to possess esterase activity not inhibited by urethan. The results presented in this paper show that urethan-grown cell material of *S. nitrificans* metabolizes a wide range of esters.

Materials and methods. Mycelium of *S. nitrificans* was grown on urethan in static cultures, harvested, washed, and homogenized as reported previously(10,12). Respiro-

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TABLE I. Metabolism of Esters by Urethan-Grown *S. nitrificans*.

Substrate, 10 μmoles/vessel	Interval,* min.	$Qo_2(N)$	% oxidation
None (autorespiration)	120	29	
Methyl formate	40	102	15
Ethyl "	50	116	17
Trimethyl orthoformate	40	266	22
Triethyl "	220	305	74
Ethyl acetate	30	169	21
n-Propyl "	70	170	19
n-Butyl "	80	162	17
Diethyl succinate	80	411	36
	190†	247	51
" malonate	110	242	34
" oxalate	60	247	24
Triethyl citrate	50	212	8
Dimethyl carbonate	40	313	44
Diethyl "	50	306	27
Ethylene "	40	311	52
Propylene "	40	285	30
Methyl cyanoacetate	110	168	64
Ethyl "	70	241	39
Methyl dichloroacetate	50	200	42
Ethyl nitrate	120‡	13	
Tetraethyl orthosilicate	40	250	9
Tributyl phosphate	120‡	44	
Benzyl cyanide	300‡	172	
Acetylcholine • HCl	300‡	6	
Choline • HCl	300‡	39	

* Unless otherwise indicated, intervals extend from time zero (substrate addition) to breaks in the curves.

† Diethyl succinate curve showed 2 breaks, one at 80 and a second after 190 min.

‡ No breaks in these curves.

metric experiments were carried out at 30°C in the conventional manner. Each Warburg vessel of approximately 16 ml capacity contained 0.1 ml of 50% KOH in the center well and 2.1 ml of fluid representing substrates and cell homogenate, equivalent to 1.4 mg cell nitrogen, in M/50 phosphate buffer supplemented with trace metals and adjusted to pH 7.0(10,12). All values for $Qo_2(N)$ have been corrected by subtracting the autorespiration.

Results. The data in Table I have been calculated from curves showing rates of gas consumption in Warburg respirometric experiments. The results reveal marked rises in oxygen uptake following addition of all substrates except ethyl nitrate, tributyl phosphate, acetylcholine, and choline. These 4 compounds caused some increases in gas consumption but to appreciably lesser extents. At the intervals indicated in Table I, rates of gas consumption decreased, *i.e.* the curves showed breaks, and tended to parallel the autorespiration except for the diethyl esters of succinate, malonate, and oxalate. With these 3 compounds, the rates of oxygen uptake, following breaks in the curves, continued to exceed that shown by the substrate-free vessel. For diethyl succinate a second break was observed after 190 minutes.

In other work reported elsewhere(8), an identically prepared cell homogenate which metabolized urethans under similar conditions was characterized by $Qo_2(N)$ values of 16, 35, 52, and 60 for 5.0 μmoles of methyl, ethyl, n-propyl, and n-butyl carbamates, respectively. Doubling the level at which these substrates were supplied did not significantly alter rate of oxygen uptake following their addition. Most esters listed in Table I were therefore metabolized from about 2 to 7 times as rapidly as the propyl and butyl urethans. The $Qo_2(N)$ for methyl formate was almost 6.5 times the corresponding value for methyl carbamate. The respective ethyl esters showed a 3.3-fold difference in $Qo_2(N)$. This variation in the reactivity for esters of carbamic acid as compared with other acids is probably not due to permeability difficulties since simple, low molecular weight, water-soluble esters generally traverse cell membranes with ease over a broad pH range(13). Furthermore, the mycelium of *S. nitrificans* used in these experiments had been homogenized by grinding(10,12).

When grown on peptone, glycerol, and yeast extract instead of urethan, the cell material of this organism showed no significant increase in oxygen uptake upon the addition of 10.0 μmoles of ethyl carbamate, for which the $Qo_2(N)$ was 0.06(10). In the present studies, however, such mycelium did show a rise in gas consumption when supplied with 10.0 μmoles each of triethyl orthoformate, $Qo_2(N) = 19$, and diethyl carbonate, $Qo_2(N) = 13$. But the corresponding values obtained with the urethan-grown preparation (Table I) were 16- and 26-fold greater. With ethyl carbamate as substrate, the $Qo_2(N) = 35$ for urethan-grown cell material(8) was 583 times the $Qo_2(N) = 0.06$ for mycelium cul-

tivated on peptone, glycerol, and yeast extract(10).

The relatively low activity of ethyl nitrate (Table I) is perplexing, since $Qo_2(N)$ values for 10.0 μmoles of ethanol alone or supplemented with 10.0 μmoles of $NaNO_3$ were 46 and 43, respectively. The increase in oxygen uptake associated with choline, $Qo_2(N) = 39$, was somewhat greater than for ethyl carbamate, $Qo_2(N) = 35$, but acetylcholine, $Qo_2(N) = 6$, showed the least effect of any ester. This reactivity of acetylcholine was of the same order of magnitude as the low acetylcholine-splitting ability observed with some bacteria(14,15). As regards choline, urethan caused a 67% inhibition in the activity of mature chicken bone marrow choline oxidase (16). But this avian tissue behaves differently from *S. nitrificans*. Urethan treatment did not reduce autorespiration or succinoxidase in chicken bone marrow, whereas both endogenous and succinate oxidation were significantly lowered by growing the actinomycete on ethyl carbamate as compared to peptone, glycerol, and yeast extract(10,12).

Of those esters which exhibited breaks in their curves, triethyl citrate and tetraethyl orthosilicate were associated with the lowest values for percentage of substrate oxidation, while triethyl orthoformate was respired to the greatest extent (Table I). In other studies with urethan-grown cell material, 17, 35, 36, and 18% of methyl, ethyl, n-propyl, and n-butyl carbamates were respectively oxidized(8). These data, however, are somewhat high since they were calculated on the assumption that the NH_2 group of the carbamate is completely converted to NH_3. Actually, some nitrite is formed(7,8,10), so that oxidation of the alcohol moiety of the esters does not account for all the oxygen consumed. This may explain at least in part the greater percentage oxidation of methyl and ethyl carbamates as compared to the corresponding formate esters. Since the urethans but not the formates can provide nitrogen for protein synthesis, one might have expected greater oxidative assimilation with the former.

Information on the metabolism of esters by actinomycetes is relatively limited. For *Streptomyces griseus* and other actinomycetes, tristearin, trilaurin, as well as vegetable and animal oils supported satisfactory growth and antibiotic production(17). Various glycolipids, phosphoglycerides, lipoproteins, cholesterol esters, acetylcholine and other esters present in myelin and human atherosclerotic aorta plaques served as growth or respiration substrates for many soil actinomycetes(18,19, 20). For a myelinolytic strain of *S. griseus*, acetylcholine allowed good cellular proliferation but increased rate of oxygen uptake only slightly in a Warburg experiment; choline, on the other hand, did not permit growth and depressed gas consumption(20). These studies and the present investigations are believed to provide the first information on acetylcholine esterase among actinomycetes.

Summary. A homogenate of urethan-grown *S. nitrificans* exhibited a marked increase in oxygen uptake when supplied with many esters. Carbamates generally exerted a lesser though significant stimulation of gas consumption. Growth on urethan therefore produced cell material containing esterase(s) characterized by broad spectrum activity. But whether carbamates are hydrolyzed by these or specific enzymes is not yet known. Acetylcholine showed the least activity of all substrates tested, although choline caused an appreciable increase in oxygen uptake.

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Milk Fat Synthesis from Acetate in Mammary Gland of the Cow. (23059)

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Previous publications from our laboratory have been concerned with the roles of short chain fatty acids, bicarbonate and glucose as precursors of milk-constituents(1-9). These have been studied by intravenous injection of various metabolites appropriately labeled with C^{14}. Acetate is the principal metabolite derived from fermentation of carbohydrates in the rumen(10), and we have shown that it is a precursor of all milk-constituents, as well as being extensively catabolised to meet energy requirements. As might be expected, plasma acetate is a potent precursor of the fatty acid moiety of milk fat, but acetate carbon is also utilized in synthesis of the glycerol moiety. In view of the large amount of acetate available to cow's tissues, this latter synthesis is probably an important source of glycerol. The evidence indicates that it takes place via the tricarboxylic acid cycle as well as via fixation of carbon dioxide derived from acetate oxidation(8,9).

The work of Popjak, Folley *et al.*(11,12) with perfused udders has shown that a considerable part of milk fat synthesis from small molecules takes place in the mammary gland itself. In this experiment 2-C^{14} labeled acetate was injected into the cistern of one quarter of the mammary gland of an intact cow, and radioactivities of milk fat components from that quarter were compared with those from the other 3 quarters and with those from a similarly labeled acetate injection made intravenously.

Methods. The technic for conducting tracer experiments with intact cows has been described by Kleiber(1). Following injection of labeled acetate, continuous samples of respired carbon dioxide were taken for the first 3 hours and then for short periods at intervals up to 35 hours. The cow was milked at 3, 10, 22, and 35 hours after injection. Details of injections and characteristics of the cows are tabulated in Table I. The fats were separated from milk samples and 4 crude fractions prepared from each. These were the glycerol, water-soluble steam-volatile fatty acids, water-insoluble steam-volatile fatty acids, and non-volatile fatty acids. Details of separation are described by Rogers (9). A sample of each fraction was combusted and the resultant carbon dioxide was trapped as barium carbonate. This was planchetted and counted at infinite thickness in a flow-gas counter. Udder injection of acetate was made into the right front quarter by passing a large blunt needle up the teat canal and depositing the acetate in the milk cistern.

TABLE I. Details of Animals Used and Isotope Injected.

	Udder inj. exp.	Intrav. inj. exp.
Wt of cow	478.5	470
Milk production, kg/day	7.4	7.6
Fat, %	4.8	5.7
Isotope inj.	1.20 mc $C^{14}H_3COOH$	3.86 mc $C^{14}H_3COOH$