cells multiplied in a medium of constant specific activity, one may calculate the rate of increase in the specific activity of DNAP if it is assumed (a) that there is no renewal of DNAP and (b) that the size of the DNAP precursor pool is negligible. After one generation, the specific activity of cellular DNAP should have reached 50% of that of the medium, after two generations 75%, and after five generations almost 100%. For Curve 1, the specific activity of the medium was assumed to be that of cellular DNAP at the end of the experiment, *i.e.*, after four generations of cells. For Curve 2, the specific activity of the medium was calculated from the measured values of total phosphorus and inorganic ^{32}P. This estimate might be low since organically bound phosphorus in the medium is probably not used as rapidly as the inorganic phosphorus. Nevertheless, the experimental points show that uptake of ^{32}P into DNA followed the expected kinetics for four cell generations and suggest that little or no renewal of DNAP occurred.

In the second type of experiment, the release of ^{32}P from the DNA of labelled cells was measured over several generations. Two Kolle flask cultures were propagated for four days at 37° C in a medium containing 40% horse serum and 2.5% chick embryo extract, in Earle's balanced saline solution, and 0.25 mc of ^{32}P as carrier-free orthophosphate. The cells were then harvested, centrifuged, washed three times in Earle's saline, suspended in Earle's saline to remove clumps, and the resulting suspension, which contained $4.6 \cdot 10^5$ cells per ml, was distributed in 0.5 ml portions into a series of T15 flasks. These flasks already contained 2.0 ml of the horse serum, embryo extract, saline mixture mentioned above. They were then incubated at 37° C, and at 2-day intervals six flasks were withdrawn for duplicate measurement of DNAP and DNA^{32}P, and for cell counts, as in the first experiment. On the fourth day, 1 ml of fluid from each of the remaining cultures was replaced with fresh medium. The results are shown in Fig. 2. There was little loss of ^{32}P from cellular DNA during multiplication in isotope-free medium.

The results of both types of experiment show that when L strain cells from the mouse multiply *in vitro* the phosphorus of the DNA fraction is not renewed to any significant extent. They also demonstrate, for this system at least, that replication of DNA does not occur in the manner postulated by STEVENS *et al.*[13] and by DAOUST *et al.*[14].

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The specific esterification of the carboxyl groups of gelatin with methanol and thionyl chloride

Carboxyl esterified gelatin was desired for studies of physical properties of modified gelatins. The alkyl sulfate and alkali[1] method could not be used as alkali causes extensive hydrolysis and as the method is not selective. The diazomethane[2] method and the methanol-acetic anhydride[3] method are also not selective. The alcohol and hydrochloric acid[4] method was successful in producing a high degree of esterification with methanol but we have found that extensive degradation occurred during the long reaction time (7 days) required. BRENNER AND HUBER[5] have reported that alcohol and thionyl chloride give good yields of amino acid esters while leaving the amino groups intact. We here report the application of the method for the first time to a protein, namely, gelatin.

The esterification of gelatin was largely complete in three hours. The amino groups were not methylated as indicated by Van Slyke amino nitrogen determination. The methoxyl content

TABLE I

ESTERIFIED GELATINS

Method	Reaction time	% Esterification		Amino** nitrogen mg/g	Intrinsic viscosity, dl/g pH 5, acetate buffer	
		Titration*	Methoxyl		0.25 M total salt***	1.25 M total salt§
Methanol-HCl	4 days	—	67	—	—	—
Methanol-HCl	7 days	67	83	—	0.19	—
Methanol-thionyl chloride	3 h	86	81	5.5	0.38	0.28
Methanol-thionyl chloride	1.5 h	60	—	—	0.48	0.38
Non-esterified		—	—	5.4	0.48	0.42

* By difference between titration curves of original and esterified gelatins in 0.1 *M* sodium chloride, using 0.1 *M* hydrochloric acid.

** Modification of DOHERTY AND OGG[7].

*** 0.1 *M* sodium acetate and 0.15 *M* sodium chloride.

§ 0.1 *M* sodium acetate and 1.15 *M* sodium chloride.

was about 6% less than that calculated from titration, indicating that methylation of other groups did not occur. By the methanol-hydrochloric acid method, the methoxyl content was greater than that calculated from titration, probably as a result of greater liberation of carboxyl groups by cleavage of peptide and amide groups. Since gelatin has few tyrosine residues[6], the possibility of etherification of phenolic hydroxyl groups was investigated using tyrosine itself. At pH 12.3, tyrosine and tyrosine subjected to the esterification treatment had ultraviolet absorption bands at the same wave length and differing by only 2% in optical density. The sulfur content of the esterified gelatin was the same as that of the starting material. The method is therefore specific for the carboxyl groups.

Some degradation may have occurred in the reaction, but much less than in the alcohol and hydrochloric acid method as is indicated by intrinsic viscosity. The properties of gelatins prepared by the two methods are shown in Table I. In the case of the esterified gelatin the reduction of viscosity on increasing the ionic strength from 0.25 *M* to 1.25 *M* was greater than for the original gelatin. This is probably due to the higher net charge on the esterified gelatin.

Experimental. An acid-extracted pigskin gelatin was used. Freeze-dried gelatin (5 g) was stirred with 200 ml of absolute methanol in a Waring Blendor and was then poured into a 3-necked flask fitted with an air-tight, motor-driven stirrer, drying tube of anhydrous calcium chloride and a dropping funnel. The flask was surrounded by an ice-salt-water bath. After one half-hour, 8 ml of water-white thionyl chloride was added dropwise during 15 minutes to the stirred mixture. After three hours in the cold bath, the mixture was filtered through a sintered glass funnel and the gelatin was washed on the funnel with four 100 ml portions of methanol. The gelatin was transferred to a beaker containing 30 ml of crushed ice, 200 ml of water was added and the jelly that formed was neutralized to pH 6 with a few drops of 2 *N* sodium hydroxide. During neutralization the jelly became fluid. The solution was dialyzed at 4° against distilled water for a week and then freeze-dried.

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