TABLE I

COUPLING OF FRACTIONS FROM ACTIVATED AND UNACTIVATED EXTRACTS

Fractions in preincubation	*Fraction added at assay time*	*Propionyl phosphate formed*
PP	PS	0.3 μM
PS	PP	0.2
PP + PS	—	2.3
PP(Act.)*	—	2.5
PS(Act.)	PP	0.2
PP(Act.) + PS(Act.)	—	2.2
PP + PS(Act.)	—	1.8
PP and PS(Act.)**	—	0.3

Conditions: The protein content of the fractions (0.2 ml of each) was as follows: PP, 1.4mg; PS, 0.6 mg; PP(Act.), 1.8 mg; PS(Act.), 0.8 mg. The fractions were incubated for 90 minutes at 30° with 20 γ of lipoic acid, 4 μM of $MgCl_2$ and 0.2 μM of cocarboxylase, and then assayed for α-ketobutyrate dismutation activity (10 minute assay), without further addition of $MgCl_2$ or cocarboxylase, but with the indicated fractions added at time of assay.

* When this fraction was assayed without preincubation, the result was the same.
** Fractions were preincubated separately and combined at time of assay.

from the activated extract could be incubated with lipoic acid and Fraction PP from the unactivated extract to reconstruct the dismutation system. Thus far, attempts to detect a heat stable or dialyzable component which could replace Fraction PS have been unsuccessful.

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The utilization of acid soluble phosphorus in growing bacteria

In studying the kinetics of desoxyribose nucleic acid (DNA) or ribose nucleic acid (RNA) synthesis, by means of ^{32}P incorporation, it is essential to ascertain whether exchange of phosphorus takes place between these substances and other sources of phosphorus within, and without, the cell.

This problem has been studied recently by HERSHEY[1] by growing labelled *E. coli* cells in cold medium and looking for a redistribution of ^{32}P between the RNA and DNA fractions. HERSHEY concluded that the conservation of ^{32}P observed in both the RNA and the DNA indicated an absence of turnover or of exchange of phosphorus within these fractions.

We have confirmed HERSHEY's results using *Salmonella typhimurium*. These results in themselves, however, are not decisive. Similar results woud be obtained if both RNA and DNA exchanged phosphorus, through a common phosphorus pool, and if the exchange rates were proportional to the net rates of synthesis of these substances[1]. However, if such exchanges occurred, the total ^{32}P-activity of the trichloroacetic acid (TCA) soluble material, of which such a common phosphorus pool may be assumed to be a part, would remain more or less constant during growth and synthesis of new nucleic acid, the return of ^{32}P from the larger, insoluble fraction maintaining an almost constant total activity in the soluble fraction. The combination of a high rate of exchange *and* loss of activity from the soluble pool, whether to the medium of to other fractions than RNA and DNA, can be excluded, since we know that the ^{32}P of the nucleic acids is conserved over several generations of growth[1].

Accordingly, a culture of *Salmonella typhimurium* was diluted to a titer of $5 \cdot 10^5$ cells/ml in

broth diluted ten-fold with distilled water, to which ^{32}P had been added, and grown to a titer of 10^8 cells/ml, at which time no further increase in ^{32}P-activity/cell was detectable. The culture was then chilled to 3°, washed free of extra-cellular ^{32}P-activity in the cold, and the cells finally resuspensed at a titer of 10^7 cells/ml and incubated at 37° (broth diluted 1:10 in 0.07 *M* phosphate buffer being used throughout). 4 ml samples were removed at intervals and treated as follows: 2 ml were frozen at once in plastic tubes immersed in a dry ice ethanol mixture. The remaining 2 ml were filtered throug a CM-Schichten bacterial filter to obtain a cell-free filtrate which was likewise frozen. The filtrate was frozen within 2 min after removing the sample. The growth of the culture was followed by means of colony counts. At the conclusion of the experiment both the total and the cell-free samples were thawed to 3°, precipitated with 5% TCA (w/v) for 1 h at 3°, carrier bacteria added, and the mixture centrifuged at 8500 r.p.m. in the Sorvall S.S.I.A. centrifuge for 15 min. 0.15 ml volumes were removed from the top of the supernatant and counted as liquid samples.

The results are presented in Fig. 1 in which the sample activities are given as a function of the number of bacterial generations.

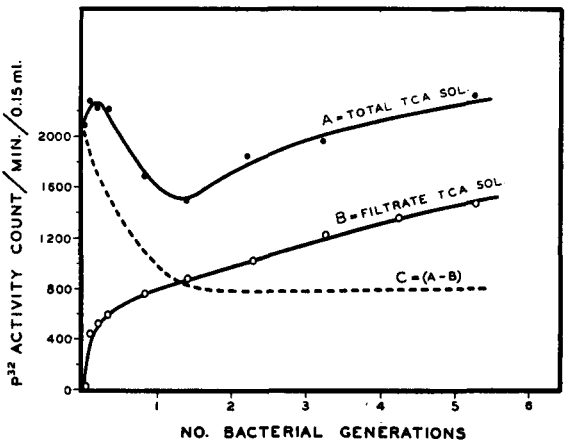

Fig. 1. ^{32}P-activity of cellular and extra-cellular TCA soluble material in a culture of *Salmonella typhimurium* as a function of the number of generations that the culture has grown at 37° in "cold" medium. Total activity of the culture was 8400 counts/min/0.15 ml liquid sample. The culture was maintained at a titer of 10^7 cells/ml during growth in the "cold" medium, by means of appropriate dilutions. Counts have been corrected for dilution. Curve A: total TCA soluble activity. Curve B: activity in TCA soluble fraction of cell-free filtrate. Curve C: difference between Curves A and B.

As may be seen in Curve B, a considerable amount of TCA soluble material appears in the cell-free filtrate with continued incubation at 37°. This was found to derive primarily from TCA insoluble material whose activity increased during the first generation from 6200 to 7100 counts/min but then fell to a value of 6500 counts/min by the fifth generation.

The ^{32}P found in the filtrate probably originates from cells undergoing autolysis as a result of injury caused by packing during the centrifugations to free the cells from medium ^{32}P. In initial experiments, it was found that attempts to separate labelled cells from the ^{32}P of the medium by centrifugation *at room temperature* resulted in the loss from the cells of up to 30% of the TCA insoluble material. No decrease in activity of TCA insoluble phosphorus is found if the cells are directly precipitated out of the labelled medium with TCA, washed and allowed to stand.

As may be seen from Curve C the intracellular TCA soluble fraction obtained as the difference between Curves A and B decreases to about 40% its initial value within the first generation and then remains constant. This initial drop, together with the constancy of the remaining activity for at least four generations, may be regarded as conclusive evidence for the absence of exchange of phosphorus between the TCA insoluble fraction and the TCA soluble fraction. The remaining 40% of the TCA soluble fraction represents in all probability inert phosphorus which does not mix with that portion of the TCA soluble pool concerned with synthetic activities. Such a fraction was described by SPIEGELMAN AND KAMEN[2] in yeast.

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