[14] Lowry, O. H., and J. A. Lopez, *J. Biol. Chem.*, **162,** 421 (1946).
[15] Fink, R. M., *et al.*, *Anal. Chem.*, **28,** 4 (1956).
[16] Lineweaver, H., and D. Burk, *J. Am. Chem. Soc.*, **56,** 658 (1934).
[17] A similar finding is reported for uracil-requiring mutants of *Schizosaccharomyces pombe* by Megnet, R., *Arch. für Vererbungsforschung, Sozialanthropologie, u. Rassenhygiene,* **33,** 299 (1959).
[18] Mitchell, M. B., and H. K. Mitchell, these PROCEEDINGS, **38,** 205 (1952).

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*ANTAGONISM BETWEEN SUBSTRATE AND REPRESSOR IN CONTROLLING THE FORMATION OF A BIOSYNTHETIC ENZYME**

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Since the formation of many degradative enzymes is induced by adding to the culture medium the corresponding substrate, it has often been suggested that the "constitutive" nature of biosynthetic enzymes depends on the endogenous formation of their substrates. It would appear possible to test this hypothesis by using a genetic block to prevent this endogenous formation. However, previous attempts to study the problem in this way[1, 2] have led to inconclusive results, since growth on the substrate was compared with growth on the end product of the biosynthetic sequence, and this end product has since been found to repress the formation of the enzyme. Furthermore, the added substrate was also serving as a source of the repressor. An additional possible complication of such experiments is formation of the substrate from the added end product, in those cases where the reactions between the two compounds are reversible.

In undertaking a reinvestigation of this problem with ornithine transcarbamylase of *Escherichia coli*, an enzyme of arginine biosynthesis which catalyzes the conversion of ornithine to citrulline, we have controlled these interfering factors by two devices: the use of a chemostat and the use of double mutants blocked both before and after the reaction under study. The chemostat made it possible to reduce repression by supplying mutants with the required arginine at low, controlled concentrations.[3] In the double mutants (Fig. 1), the earlier block

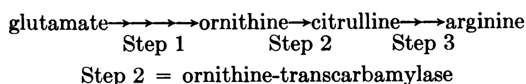

FIG. 1.—Schematic pathway of arginine biosynthesis.

prevents endogenous formation of the substrate (ornithine), while the later block prevents both formation of the end product of the seqence from added substrate and formation of the substrate from added end product. In *E. coli* there appears to be no additional pathway to ornithine.[4] The study has been extended to include not only arginine-repressible strains but also strains in which arginine does not have this effect.

Bacterial Strains.—In the present experiments the wild type and mutants of different strains of *E. coli* have been studied: the W strain, in which added arginine

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represses the formation of ornithine transcarbamylase, and two variants of the B strain, in which it does not. These two variant strains are: normal B, which produces low concentrations of the enzyme, and B(4S-7), derived from B[5], in which the level of the enzyme is 40 times higher.†

Table 1 contrasts the effect of added arginine on the enzyme levels in repressible strains (W and K12), which may be designated as R+ (R for repressibility), and

TABLE 1

LEVEL OF ORNITHINE-TRANSCARBAMYLASE (U/MG BACT. DRY WEIGHT) AT DIFFERENT LEVELS OF ARGININE POOL IN REPRESSIBLE AND NONREPRESSIBLE STRAINS

		Enzyme Level		
Strain	Repressibility	Arginine pool very low*	Arginine pool normal†	Arginine pool high‡
K12	+	104.0	8.0	0.5
W	+	67.0	2.0	0.1
B	−	4.0	5.0	7.0
B(4S-7)	−	140.0	132.0	160.0

* Arginine-requiring mutant, blocked in position 1 or 3, growing in a chemostat under arginine limitation.[9]
† Wild type exponentially growing in minimal medium A.
‡ Wild type or arginine auxotroph growing in excess of arginine.

in nonrepressible (R−) strains [B and B(4S-7)]. The designation of repressibility as a genetic character distinct from enzyme formation is based on the result of experiments in which mutants lacking the enzyme (E−) were derived from the nonrepressible B or B(4S-7) strains and crossed with a repressible E+ donor of the K12 strain.[5] Two classes of E+ recombinants were obtained: R+ and R−.

To obtain the desired double mutants (designated as type 1–3) of the various prototrophic strains, one-step mutants blocked somewhere before ornithine (block 1) were irradiated with ultraviolet light and second step mutants were selected which could grow on arginine but no longer on citrulline (block 3). The selection was carried out by a modification[6] of the penicillin technique which improves the recovery of rare auxotrophic mutants. The retention of block 1 in each 1–3 mutant was verified by checking the requirement of back mutants, selected on ornithine, that had lost block 3.

Growth Conditions.—The organisms were grown at 37° in mineral-glucose-citrate medium A,[7] supplemented with arginine, as necessary. The chemostat experiments followed the usual procedure.[3]

Determination of Ornithine-Transcarbamylase Activity.—Enzyme activity was determined in toluenized cells as previously reported.[8] One unit of enzyme is the amount which synthesizes 1 μmole of citrulline per hour. The figures reported in the tables are units of enzyme per mg bacteria (dry weight).

Control of Intracellular Arginine Levels.—It has been shown previously[9] that in the W strain of *E. coli* growth in the presence of exogenous arginine results in essentially complete repression of ornithine transcarbamylase, i.e., the level of this enzyme is reduced to a barely detectable value. On the other hand, the maintenance of an abnormally low intracellular arginine level, by slow growth of an arginine auxotroph in an arginine-limited chemostat, results in a concentration of the enzyme 25 to 50 times that found under "normal" conditions (i.e., in the wild type growing on minimal medium).[9] This state has been called derepression. For purposes of the present work it was necessary to see whether the chemostat

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could be used to maintain arginine levels that would result in enzyme concentrations intermediate between complete repression and derepression.

In the chemostat,[3] with an arginine auxotroph and a medium in which arginine limits the density of growth, differences in the rate of flow of new medium are associated with parallel differences in the steady-state rate of growth. This remains true over a range of growth rates corresponding to division times between one hour and several hours. At higher flow rates, exceeding the division time of the organism without limitation of arginine, the population density falls progressively and arginine accumulates rapidly in the culture fluid. Under steady-state conditions, since arginine is limiting, the theory of the chemostat[3] predicts that each growth rate is associated with a characteristic extracellular (and therefore a characteristic intracellular) level of free arginine: the shorter the division time, the higher the arginine level.

It should thus be possible to run the entire gamut of intracellular arginine concentrations by varying the flow rate in the chemostat. Figure 2 shows the effects of such variation on the concentration of the enzyme in mutant 160–37 of the W strain of *E. coli*, which is blocked before ornithine. It is seen that over a range of low flow rates there is complete derepression—i.e., maximal enzyme formation. At the other extreme, at flow rates too great for arginine limitation, there is essentially complete repression, with very low levels of the enzyme similar to those seen in the wild type growing in the presence of excess arginine. At flow rates approaching the limit of the steady-state (1–1.5 hr division time) intermediate levels of the enzyme are seen. It is thus possible by use of the chemostat to maintain in an arginine auxotroph levels of the enzyme (and hence intracellular levels of arginine) similar to those produced by the wild type growing on minimal medium.

Effect of Ornithine in a Repressible Strain.—The effect of ornithine on transcarbamylase formation was studied in a type 1–3 mutant of an R+ strain. It is known[9] that in R+ strains arginine represses transcarbamylase synthesis irrespective of any genetic block interposed in the sequence of enzymes leading to arginine synthesis. In order to control the level of arginine the experiments were performed in chemostats with arginine-limiting growth. Two situations have been analyzed: partial repression and derepression. The effect of different concentrations of ornithine has been tested under each of these conditions.

To achieve the partially repressed condition, the experiment was started at a flow rate as fast as was compatible with the steady state. As is seen in Figure 2, these conditions resulted in an intracellular arginine level that repressed the enzyme content per cell to about $^1/_{20}$ of its maximum, i.e., similar to that in wild-type strain W growing exponentially in minimal medium. At the end of the experiment, in order to achieve the condition of derepression, the intracellular arginine level was lowered by halving the flow rate. As is seen in Figure 2, under these conditions a maximal level of enzyme is reached, i.e., maximal derepression. The enzyme level was determined in samples taken after each steady state was reached, i.e., 6 to 8 hours after the start of the experiment, after the addition of ornithine, and after the change in flow rate. That a steady state was reached is shown by the fact that samples taken 3 hours later gave essentially the same values.

Tables 2 and 3 give the results of two typical experiments. It is seen that under a condition of partial repression (fast flow rate) ornithine stimulates formation

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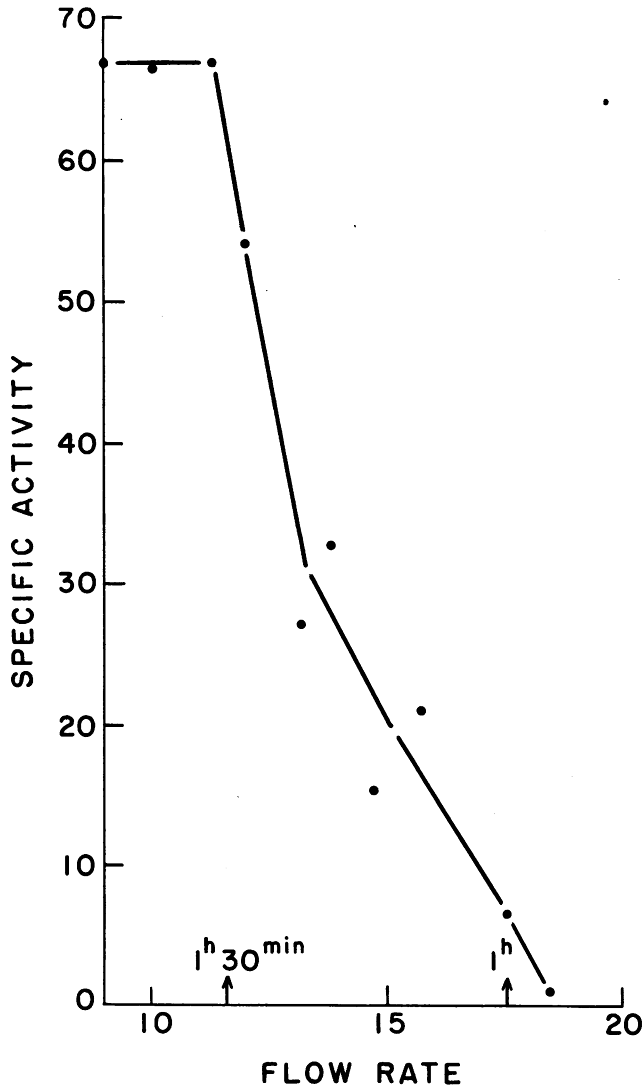

FIG. 2.—Enzyme level in a repressible strain versus rate of flow. Mutant W160-37, type 1-3, was grown in a chemostat under arginine limitation. The culture was fed with medium containing 10 mg of arginine/L at the flow rate indicated in the abscissa (ml/hr). The ordinate is specific activity: units of ornithine-transcarbamylase/mg bacteria dry weight. The arrows on the abscissa indicate the division time calculated from the flow rate (W) and the total volume of the culture ($V = 25$ ml) according to the formula: div. time = $(V/W) \times ln2$. Steady-state conditions are possible only with flow rates lower than 17.5 ml/hr corresponding to a division time of 1 hr.

of the enzyme.‡ Ornithine totally overcomes this degree of repression at a concentration of 15 to 20 μg/ml in the input medium. Furthermore, by comparing the two tables one realizes that the stronger the repression, the higher is the concentration of ornithine required in the input medium to yield a given level of the enzyme

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TABLE 2

EFFECT OF ORNITHINE ON LEVEL OF ORNITHINE-TRANSCARBAMYLASE (U/MG BACT. DRY WEIGHT) IN AN R+ STRAIN BLOCKED IN POSITIONS 1 AND 3

Chemostat Experiment: Arginine Limiting

	Enzyme Level at Ornithine Conc. of:			
Flow rate	0 μg/ml	1 μg/ml	10 μg/ml	100 μg/ml
Fast (=1 hr div. time)	5.1(6.3)	21.9(22.0)	50.5(42.0)	61.5(67.0)
Slow (2 hr div. time)	50.0(60.0)	56.2(63.0)	50.5(54.0)	58.0(51.0)

Organism.—Mutant W 160–37 blocked between acetylornithine and ornithine (block 1) on which has been imposed a second block between citrulline and arginine (block 3).
Growth conditions.—Medium A with 0.1% glucose and 10 μg/ml of arginine. Four parallel chemostats with identical flow rates. Ornithine is added to the input medium at time zero.
The figures in parentheses are the levels of enzymes in samples taken 3 hr after the initial sample. They demonstrate that steady state had been established.

TABLE 3

EFFECT OF ORNITHINE ON LEVEL OF ORNITHINE-TRANSCARBAMYLASE (U/MG BACT. DRY WEIGHT) IN AN R+ STRAIN BLOCKED IN POSITIONS 1 AND 3

Chemostat Experiment: Arginine Limiting

Flow rate	Addition of Ornithine	Enzyme Level: Chemostat 1	Enzyme Level: Chemostat 2
Fast (= 1 hr/div. time)		2.4(4.5)	3.5(2.5)
	→	+2μg/ml	+20μg/ml
Same rate		20.0(15.8)	42.5(47.0)
Slow (2 hr div. time)		56.0	58.0

Organism.—The same as in Table 2.
Growth Conditions.—Same medium as in Table 2.
Two parallel chemostats with identical flow rates. Ornithine is added to the input medium after equilibration of the chemostat.
The figures in parentheses are the levels of enzymes in samples taken 3 hr after the initials ample. They demonstrate that steady state had been established.

(1 μg of ornithine per ml to raise the enzyme from 5.1 to 21.9 units in Table 2, as compared with 2μg of ornithine per ml to raise it from 2.4 to 15.8 units in Table 3). In the extreme case of a fully repressed culture (growing in a flask with an excess of arginine) 1,000 μg of ornithine per ml (about 15 times the concentration of arginine) are required to obtain a detectable reversal of the repression. At the other extreme, in a completely derepressed culture (slow flow rate, Tables 2 and 3), ornithine had no effect on the level of the enzymes. These results suggest that the stimulatory effect of ornithine on the enzyme level depends on competitive antagonism to the repressive effect of arginine.

Effect of Ornithine in Nonrepressible Strains.—The effect of ornithine has also been studied in strains in which arginine does not repress the synthesis of transcarbamylase. Since control of arginine level during growth did not seem important with these strains, the experiments were carried out in flask cultures and the level of the enzyme was measured after overnight growth. However, in order to be sure that the arginine level did not affect the results, two extreme conditions have been analyzed: arginine limitation and arginine excess.

It is seen (Table 4) that with these R− strains addition of ornithine was not required for, and furthermore did not stimulate, formation of the enzyme, even in the presence of excess arginine. It should also be noted that the constant values of this enzyme as seen in these mutants under these various conditions are comparable to those observed in the prototrophic strains (Table 1) from which those double mutants were derived.

The results obtained with R+ and R− strains suggest that ornithine stimulates the formation of transcarbamylase only in the presence of arginine.

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TABLE 4

EFFECT OF ORNITHINE ON LEVEL OF ORNITHINE-TRANSCARBAMYLASE (U/MG BACT. DRY WEIGHT) IN R− STRAINS BLOCKED IN POSITIONS 1 AND 3

	Enzyme Level			
	Arginine limiting*		Arginine in excess†	
In presence of ornithine:	Strain B-(1–3)	Strain B(4S-7)-(1–3)	Strain B-(1–3)	Strain B(4S-7)-(1–3)
0	5.2	135.0	7.5	169.9
10 μg/ml	4.9	138.0	..	...
60 μg/ml	..	...	8.0	164.0
100 μg/ml	..	...	7.2	165.0

Organisms.—Type 1–3 mutants of B and B(4S-7).
Growth Conditions.—Overnight cultures, growth limited by: * Arginine (arginine 20 μg/ml); † glucose (arginine 60 μg/ml).

Nonrequirement of Ornithine in the Absence of Repression.—A double block in positions 1 and 3 should prevent endogenous formation of ornithine. However, such a mutant is ornithine-free only if block 1 is complete. Indeed, the effect of any leakage in block 1, even if undetectable by growth tests, would be enhanced by the impositions of block 3 because any ornithine formed would tend to pile up. It would therefore be desirable to obtain further evidence to determine whether or not traces of endogenous ornithine may be responsible for the formation of the enzymes under conditions of derepression.

For this purpose, mutants blocked only in position 1 and mutants blocked only in position 3 were grown in a chemostat at a slow rate under arginine limitation, and their enzyme levels were compared. Under these conditions the intracellular level of endogenous ornithine should be high in mutants blocked in position 3 and very low, if not zero, in mutants blocked in position 1.

Table 5 gives the enzyme level in Type 1 and Type 3 mutants of several strains (including R+ and R−). It is seen that in every strain under these derepressed conditions the enzyme level in mutant 1 is not significantly different from that in mutant 3. These results, together with those reported above for type 1–3 mutants, support the conclusion that in the absence of repression, whether because the strain is not repressible or because insufficient repressor is present, ornithine is deprived of any influence on the formation of transcarbamylase. Furthermore, the results presented in Table 5 show that whatever may be the factor responsible for the differences in production of this enzyme by the different strains, it is not the level of endogenous ornithine.

TABLE 5

LEVEL OF ORNITHINE-TRANSCARBAMYLASE (U/MG BACT. DRY WEIGHT) IN MUTANTS OF VARIOUS STRAINS BLOCKED IN DIFFERENT POSITIONS

		Enzyme Level		
Parent strain	Repressibility	Block 1*	Block 3*	Wild type†
W	+	65.0	62.0	...
K12	+	94.0	100.0	...
B(4S-7)	−	150.0	146.0	145.0
B	−	6.0	7.0	5.0

* Cultures growing in a chemostat under arginine limitation (10 μg/ml). Division time = 4 hr.
† Overnight culture in minimal medium A.

Discussion.—By using double mutants blocked both before and after ornithine transcarbamylase, and by controlling the arginine concentration by means of growth in a chemostat, it has been found that ornithine can reverse the repressive

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effect of arginine on the formation of this enzyme. The induction by ornithine occurs only under conditions of partial repression. When repression is eliminated by sufficiently lowering the arginine concentration, or by using a nonrepressible strain, the inducing effect of ornithine is also eliminated and the concentration of the enzyme for the given strain appears to be maximal without any endogenous or added ornithine. Conversely, when repression is complete, in the presence of excess arginine, even comparatively large amounts of ornithine are without effect. The reversing effect of ornithine appears to be competitive with arginine. However, a strict test for competition was not possible because of technical difficulties in controlling intracellular arginine levels over a sufficient range.

Because the repressor and its antagonist are both provided exogenously in these doubly blocked mutants, one must consider the possibility that the observed interactions might be based simply on competition for entry into the cell. Owing to this consideration, the results of preliminary experiments performed in flask cultures were inconclusive. In these experiments the effect of ornithine on the level of transcarbamylase was studied in overnight cultures whose growth was limited by arginine. Five to six times more enzyme was found in the presence of ornithine than in its absence. However the possibility that this effect was due to arginine-ornithine competition for entry could not be ruled out.

As is shown by the following reasoning, the present experiments in the chemostat have eliminated the possibility that the inducing effect of ornithine could be due to interference with the entry of arginine. Since the growth rate of an arginine-limited culture is determined by the intracellular concentration of arginine, each steady-state flow rate determines a corresponding fixed intracellular concentration. Under ordinary conditions, in the absence of any inhibitor of entry, each steady-state intracellular concentration will correspond to a fixed extracellular concentration. When a competitive inhibitor of entry is added to the medium in a steady-state system the entry of arginine will be slowed and the cell density will decrease, until the external arginine concentration reaches a new higher level which precisely compensates for the impaired membrane transport. At this point a new steady state will be reached, with restoration of the original division time and hence the original intracellular arginine concentration. It follows that if ornithine should interfere with arginine entry it would not affect the intracellular level of arginine in these experiments, provided enough time is allowed for reaching the steady state.

Actually, at a fast flow rate the extracellular arginine is an appreciable fraction of the total amount available, and so competition for entry might be expected to cause a measurable drop in cell density. In our experiments the drop observed after addition of ornithine was never more than $^1/_{10}$ of the original density, indicating that the competition for entry is small.[§] Hence, the time allowed in these experiments (several generations) was ample for reaching the new steady state, as is verified by the essentially constant values of the enzyme found during an additional interval of 3 hrs (Tables 2 and 3).

It seems safe to conclude that the inducing of ornithine is based on competition with arginine at some site within the cell: either the site of repressor action, if arginine is the direct repressor, or the site of repressor formation, if arginine is a precursor of the direct repressor. Since transcarbamylase is necessarily present

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in these experiments, the possibility cannot be rigorously excluded that citrulline rather than ornithine is the true reverser of repression. However, since the reaction is reversible, either compound is a substrate of the enzyme, and the phenomenon would still be one of induction of a biosynthetic enzyme by its substrate.

Recent work on the β-galactosidase system[10] has strongly indicated that the mode of action of the inducer is to counteract repression by an unknown compound, an inducible strain thus being actually R+ and a constitutive strain (which does not require any inducer) being R−. The present findings provide another kind of evidence, in which the repressor is known, in support of the same conclusion for another enzyme. Furthermore, by showing that a biosynthetic enzyme can respond to an inducer as well as to a repressor, these findings furnish further evidence against the earlier idea that there might be any fundamental difference in the mechanism or in the control of formation of so-called constitutive and adaptive enzymes. At the same time, since ornithine is an inducer only under conditions where arginine is acting as a repressor, these findings eliminate, at least for this system, the often-quoted speculative proposition that the constitutive nature of biosynthetic enzymes might be due to the inductive effect of the endogenously formed substrate.

Summary.—The effect of ornithine on the synthesis of ornithine-transcarbamylase has been studied in strains (R+) in which the synthesis of this enzyme is repressed by arginine and in strains (R−) in which it is not repressed.

To permit independent control of ornithine and arginine concentration, double mutants blocked both before and after this enzyme were used. Furthermore, the chemostat was used to maintain arginine levels that resulted in enzyme concentrations intermediate between complete repression and derepression.

Ornithine reversed the repressive effect of arginine in an intermediate concentration range; however, it was without effect under conditions of complete repression or derepression.

The nature of the chemostat experiment is self-correcting for arginine-ornithine competition for entrance into the cell. It therefore appears that ornithine competes directly with arginine at some site within the cell.

An analogy exists between the repressor-inducer relationship in the cases of ornithine transcarbamylase, a biosynthetic (so-called "constitutive") enzyme, and β-galactosidase, a degradative (so-called "inducible") enzyme.

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† Genetic recombination experiments, which will be discussed elsewhere, indicate that this difference in level of enzyme activity is due to the existence of a controlling factor genetically distinct from repressibility, rather than to an alternation in the efficiency of the enzyme formed. The action of ornithine has been tested in both variant strains in order to test a possible interaction of ornithine with such a control mechanism.

‡ Since it is difficult to maintain rigorous steady-state conditions in the chemostat at the fast flow rates of these experiments, which approach the threshold for escape from the steady state, one must consider the possibility that the apparent effect of ornithine on the level of the enzyme might be due, in reality, to uncontrolled fluctuations in the flow rate. However, one can estimate from Figure 2 that the flow rate would have to change by a factor of 1.5 in order to obtain the

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maximal effect ascribed to ornithine in Tables 2 and 3. This change would far exceed the possible error in the control of flow rate in these experiments. It follows that the observed inducing effect of ornithine is a real one, whatever its mechanism.

§ Further support of this conclusion has been obtained in unpublished experiments of Dr. I. B. Weinstein. *E. coli* W160-37 type 1-3 was depleted of arginine, treated with chloramphenicol (200μg/ml) to prevent protein synthesis, and incubated for 5 min with C^{14}-arginine (10 μg/ml) in the absence and in the presence of an excess of unlabeled ornithine (500 μg/ml). The results showed that ornithine did not interfere with the uptake of arginine.

[1] Vogel, H. J., and B. D. Davis, *Federation Proc.*, **11**, 485 (1952).
[2] Wijesundera, S., and D. D. Woods, *Biochem. Jour.*, **55**, viii (1953).
[3] Novick, A., and L. Szilard, *Science*, **112**, 715 (1950); *Dynamics of Growth Processes* (Princeton: Princeton University Press, 1954), p. 21.
[4] Roberts, R. B., *et al.*, *Studies of Biosynthesis in Escherichia coli* (Carnegie Institution of Washington, D. C., 1955), p. 160.
[5] Gorini, L. (unpublished results).
[6] Gorini, L., and H. Kaufman, *Science*, **131**, 604 (1960).
[7] Davis, B. D., and E. S. Mingioli, *Jour. Bacteriol.*, **60**, 17 (1950).
[8] Gorini, L., *Bull. Soc. Chim. Biol.*, **40**, 1939 (1958).
[9] Gorini, L., and W. K. Maas, *Biochim. et Biophys. Acta*, **25**, 208 (1957).
[10] Pardee, A., F. Jacob, and J. Monod, *Jour. Mol. Biol.*, **1**, 165 (1959).

THE REPLICATION OF DESOXYRIBONUCLEIC ACID IN HEPATOCYTES

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The process of replication of DNA in hepatocytes in rats following partial hepatectomy has been extensively studied by biochemical methods;[1-9] however, one of the major limitations of the biochemical methods is that it has not been possible to determine the time sequence of replication of the DNA in individual hepatocytes because of the changing population of hepatocytes synthesizing DNA.

Individual hepatocytes have been followed throughout replication of the DNA content by labeling nuclei with tritiated thymidine and measuring the DNA content microspectrophotometrically at varying times after administration. Quantitative autoradiography and microspectrophotometry have given independent methods for the measurement of the relative changes of DNA during replication in the same nuclei.[10, 14, 20]

Materials and Methods.—Combined autoradiographic and cytochemical studies have been carried out on rats sacrificed between $18^1/_2$ and 26 hr after partial hepatectomy. The weights have been between 200–300 grams and the age between 4–5 months. All rats were given intravenously 50 μc of tritiated thymidine (either 1.9 or 3 curies per millimol) diluted to a volume of 0.5 cc in normal saline.

The regenerating livers were minced with scissors and mixed. Random specimens of these small pieces of liver were taken and gently pressed between cover slips, followed by immersion in liquid propane. The specimens were quickly transferred to ethyl alcohol (cooled to −78 degrees C by solid CO_2).[11] All liver speci-

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