TABLE I. Tumors Induced in Albino Mice with 2-Acetylaminofluorene (AAF), 6 Groups.

Treatment	Total No. mice*	Sex	Observation period (avg days)	Mice with tumors: Hepatomas	Mice with tumors: Pulmonary adenomas
AAF - suspension	62	♂	365	37	17
Idem	86	♀	362	1	27
Suspension only	27	♂	364	1	8
Idem	25	♀	366	0	9
None	29	♂	365	1	10
"	28	♀	365	0	9

* Survivors at time first hepatoma was observed.

of treatment were selected arbitrarily, it is possible that an even smaller amount of AAF, and given over a shorter time, also may be effective. These results demonstrate that 7-8 day old male mouse is highly susceptible to hepatocarcinogenic action of orally administered AAF.

Summary. Administration by stomach tube of a suspension containing 2-acetylaminofluorene to suckling albino mice was followed by development of high incidence of hepatomas in males (60%) but not in females (1%). A similarly low incidence of hepatomas (0%-4%) was observed in untreated mice from same strain or mice treated with suspension alone. The low dose of 2-acetylaminofluorene represents smallest quantity of this compound effective to date in liver tumorigenesis. Also, results indicate that suckling male mouse is especially susceptible to hepatoma induction with 2-acetylaminofluorene.

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Transamination in *Leptospira biflexa.** (25044)

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Few studies on metabolism of leptospirae have been published. Chang(1) reported that sugars were not utilized by *Leptospira icterohemorrhagiae.* Marshall(2) and Helprin and Hiatt(3) studied the effect of serum or fatty acids on oxygen utilization by whole cells of *L. icterohemorrhagiae.* Fulton and Spooner (4) using the same organism analyzed the growth medium for chemical changes and found that small quantities of volatile fatty acids accumulated. An investigation of amino acid metabolism in the leptospirae was undertaken since sugars are not utilized(1). Enzymatic transamination, firmly established in animal tissue and other bacteria(5), was performed with whole cells and cell-free extracts of *L. biflexa.*

Materials and methods. The culture of *L. biflexa* was obtained from A. D. Alexander, Walter Reed Army Medical Center. Leptospirae were grown in 4 l quantities in medium of Cox(6) and harvested by centrifugation

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TABLE I. Transaminations by Cell-Free Extracts of *L. biflexa.*

	mg dry cell ext./ml	Amino donor,† μmoles/ml		Amino acceptor, μmoles/ml	Amino acid formed, μmoles/ml
(a)				α-ketoglutarate	Glutamic acid
	5.8*	None	0	0	0
	"	"	0	16.6	0
	"	DL-Aspartic	16.6	"	1.70
	"	" -Isoleucine	"	"	2.20
	"	" -Leucine	"	"	4.78
	"	" -Methionine	"	"	2.38
	"	" -Phenylalanine	"	"	4.21
	"	" -Tryptophan	"	"	3.49
	"	L -Tyrosine	8.3	"	1.05
	"	" -Valine	"	"	.71
(b)				Pyruvate	Alanine
	4.1	None	0	0	0
	"	"	0	18.2	0
	"	DL-Isoleucine	18.2	"	2.92
	"	" -Leucine	"	"	1.46
	"	L -Glutamic	9.1	"	3.93
(c)				Oxalacetate	Aspartic acid
	5.7	None	0	0	0
	"	"	0	21.7	0
	"	L-Glutamic	10.9	"	6.24

* In all cases the numbers listed represent final concentration.
† Only amino acids showing transamination listed. For those used see text.

after 9 to 12 days incubation at 30°C. Cells were washed 3 times in 0.1 M phosphate buffered saline (pH 7.2) and suspended finally in the same menstruum. Cell-free extracts were prepared by sonic oscillation and freed of cellular debris by centrifugation at 15,000 x G for 15 minutes. Dry weights of cell-free extracts varied between 10 and 20 mg/ml. Cell-free extracts were dialyzed against 10^{-5} M phosphate buffer (pH 7.2) for 24 hours before use to remove glutamic acid present in small quantities. L or DL amino acids were used as amino donors and concentrations of amino acids were doubled when DL mixtures were used. The amino acids tested for transamination included DL-alanine, DL-aspartate, DL-arginine, L-glutamate, glycine, L-histidine, DL-isoleucine, DL-leucine, DL-lysine, DL-methionine, DL-phenylalanine, L-proline, DL-serine, DL-threonine, DL-tryptophan, L-tyrosine, and L-valine. In experiments in which glutamate, aspartate, or alanine was produced by transamination, the respective amino acid was not included as amino donor. Keto acid, amino acid, cells or dialyzed cell-free extract, and phosphate buffer (pH 7.2, 0.01 M final concentration) were incubated in test tubes under anaerobic conditions for 150 minutes at 30°C. Reactions were terminated by placing tubes in boiling water bath 5 minutes and removing precipitated cells or protein by centrifugation. Ascending paper chromatography in conjunction with colorimetric procedure of Housewright and Thorne(7) was used to measure quantity of amino acid produced.

Results. A preliminary experiment was performed using α-ketoglutarate as amino acceptor in presence of whole cells. DL-aspartate, DL-isoleucine, DL-leucine, DL-methionine, DL-phenylalanine, DL-tryptophan, L-tyrosine, and L-valine transaminated with α-ketoglutarate to form glutamate.

Since transamination reactions were catalyzed by whole cells, further experiments were done with dialyzed cell-free extracts. Table I shows results obtained with α-ketoglutarate, pyruvate, or oxalacetate as amino acceptors. Absence of amino acid formation in controls demonstrates that amino acids were not produced by non-specific means. Eight amino acids transaminated with α-ketoglutarate, 3 with pyruvate and only glutamate with oxalacetate.

Table II presents results of experiment performed to demonstrate the role of pyridoxal

TABLE II. Effect of Dialysis on Glutamic-Aspartic Transaminase.

Additions incubated at 30°C for 70 min.		Glutamate formed, μmoles/ml
Aspartate	18.5 μmoles/ml*	2.21
α-ketoglutarate	*Idem*	
Undialyzed cell-free ext.	8.8 mg/ml	
Aspartate	16.6 μmoles/ml	1.70
α-ketoglutarate	*Idem*	
1×10^{-5} M phosphate buffer dialyzed cell-free ext.	5.8 mg/ml	
Aspartate	18.5 μmoles/ml	0
α-ketoglutarate	*Idem*	
Versene dialyzed cell-free ext.	4 mg/ml	
Aspartate	15.6 μmoles/ml	.51
α-ketoglutarate	*Idem*	
Versene dialyzed cell-free ext.	4 mg/ml	
Pyridoxal phosphate	12.5 "	

* In all cases the numbers listed represent final concentration.

phosphate in transamination reactions of *L. biflexa*. Dialysis of cell-free extract against 10^{-5} M phosphate buffer (pH 7.2) even if extended over a long period of time did not eliminate aspartic-glutamic activity. Dialysis of cell-free extracts against versene according to procedure of Racker, *et al.*(8), followed by dialysis against distilled water to remove versene, resulted in complete loss of transamination activity. Activity was partially restored by addition of pyridoxal phosphate.

Discussion. Whole cells and cell-free extracts gave similar results when α-ketoglutarate was the amino acceptor. Leptospirae require oxygen for growth and do not utilize sugars. Consequently, no apparent means of energy metabolism transpires to facilitate transportation of keto acid or amino acid into the cell. It seems unlikely that both amino acid and keto acid are passively permeable.

In general, transamination reactions of *L. biflexa* are similar to those known to occur in other microorganisms, plant and animal tissues(5). However, different amino acids are active in leptospiral transamination in comparison to reactions in another spirochete, the Reiter treponeme(9). The capacity of cell-free extracts of *L. biflexa* to carry out a number of transamination reactions emphasizes an active amino acid metabolic system whether or not such reactions are involved in synthesis or degradation. That leptospirae possess cytochrome(4), and are able to metabolize amino acids to some extent as shown here, indicates that amino acids should be considered as possible energy sources along with fatty acids(3). Further studies on oxidative deamination of amino acids by leptospirae and determination of citric acid cycle enzymes would be very important in this regard.

Most reactions studied were not checked for reversibility. However, the glutamic-aspartic reaction was reversible. Attempts to demonstrate reversibility of glutamic-alanine transamination reaction were not successful, although several experiments were performed with varying concentrations of alanine. We can give no explanation of these results.

That pyridoxal phosphate is the coenzyme for transamination was expected. It is of interest that dialysis against versene resolved the system. Perhaps greater reactivation could have been attained by addition of magnesium ions along with pyridoxal phosphate (10).

Summary. Whole cells and cell-free extracts of *Leptospira biflexa* have been used to demonstrate a series of transamination reactions. Dialysis against versene was used to show that pyridoxal phosphate served as a coenzyme. The possible importance of transamination in leptospiral metabolism is discussed.

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