ships and possible antagonisms might develop between various groups of growth factors. The only instance of such effects encountered up to the present time is that between glutamine and ATP plus adenylic acid. No explanation of this interesting relationship is available at the present time.

The necessity of cultivating the tissues in naturally-occurring nutritive media for 3 to 5 days prior to the replacement of these natural media by synthetic mixtures is an undesirable feature of the present experiments. During this preliminary period of cultivation, the cells might be able to store up reserve food substances that would serve as a source of nutrient during the subsequent period of cultivation in synthetic media. Still, tissues cultivated in Earle's solution alone degenerated and died within 6 or 7 days (Table I). This would seem to indicate that the effects of the initial period of maintenance in the preliminary feeding mixture are not exerted long after this original fluid is removed. Eventually it is hoped that this early maintenance period in non-synthetic media may be eliminated.

The systematic addition of new ingredients to the synthetic mixture has seldom resulted in a marked improvement that could be attributed to any one substance. But periodic comparisons of the effect of the more recent test solutions with simpler mixtures that had been used earlier (Table I) have shown a progressive increase in the survival time of the cultures and a marked improvement in their appearance. Thus, for example, results obtained with Mixture 199 were vastly superior to those obtained with mixtures containing only amino acids, vitamins and Tween 80.

The results reported in the present communication have established that synthetic Mixture 199 will support the survival of chick embryo tissues for an average period of 4 to 5 weeks. Although this mixture will not support the cells indefinitely, it does appear suitable for use as a basal synthetic medium for further investigations on cell nutrition.

Summary. The nutrition of animal cells in tissue culture has been studied with particular reference to amino acids, vitamins, nucleic acid constituents and various accessory growth factors. The optimal concentrations of these substances for the maintenance of cell life *in vitro* have been established and a completely synthetic feeding solution has been devised. Although this mixture will not support cell life indefinitely, it has been found adequate to maintain chick embryo cells for an average period of 4 to 5 weeks. The mixture is being used as a basal synthetic medium for further studies on the unidentified growth-promoting substances known to occur in natural media.

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III. Influence of Renal Tissue in Inhibition of Protein Catabolism.* (17558)

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Data are presented elsewhere(1) which show that the rate of protein catabolism, as measured by the rate of urea formation, is increased markedly in rats after sudden loss of 75% or all of the total renal tissue. A higher rate of protein catabolism was found in rats subjected to complete nephrectomy than in those with 25% of the total renal substance remaining. It was suggested that loss of 75% or all of the total renal tissue might release the mechanism responsible for

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1. Persike, E. C., and Addis, T., *Am. J. Physiol.*, 1949, v158, 149.

breakdown of body protein from one of its usual inhibitory influences. The experiments reported here support this hypothesis.

Methods. The experimental conditions and methods, and data obtained from control animals are given in detail elsewhere.(1) One hundred and fifty female albino rats, selected at body weights of 150 g, were divided into groups of 10 rats each. The rats of 9 groups were submitted to total nephrectomy. Bilateral ureteral ligations, with division of the ureters between ligatures, were performed on the rats of 6 groups. The rats were fed a caloric-ally adequate solution of 15% glucose in water with 0.4% sodium chloride and vitamins of the B complex for 2 days before operation, and after operation until autopsy, in order to eliminate the influence of food protein consumption on the serum urea concentrations. Three groups of nephrectomized animals were killed at the end of the 4th, 16th and 24th postoperative hours, and 3 groups of rats subjected to bilateral ureteral ligation were killed at the end of the 12th and 24th postoperative hours. The animals were killed by exsanguination under ether anesthesia, and aliquot portions of serum were combined to form one serum pool for each group. Serum urea concentrations were determined by the urease aeration method of Addis,(2) all measurements being made in duplicate. Rates of urea formation were calculated by the procedure used in other experiments.(1)

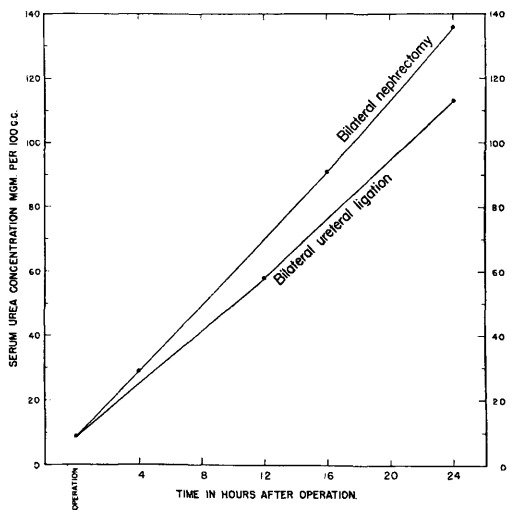

FIG. 1.
Average serum urea concentrations.

Results. Fig. 1 shows that the serum urea concentrations both of the nephrectomized animals and of the rats subjected to ureteral ligation continuously increased after operation until the end of the 24th postoperative hour. The serum urea concentration of the nephrectomized rats, however, increased at a more rapid rate. At the end of the 24th postoperative hour it exceeded the serum urea concentration of the rats whose ureters were ligated by 22.7 mg per 100 cc, or approximately 20%.†

The rates of urea formation are given by Table I. In each successive postoperative 4 hour period, the rate of urea formation of the nephrectomized rats was greater than that of the animals subjected to bilateral ureteral ligation. The total amount of urea formed during the 24 postoperative hours was 120 mg per rat by the nephrectomized animals, and 99 mg per rat by the rats with ligated ureters. The magnitude of this difference cannot be explained by supposing inequalities in hydration, because both types of rats gained an equal amount of weight after operation, and presented grossly equal quantities of subcutaneous and intraperitoneal fluid at autopsy.

Discussion. While the excretory function of the kidneys ceases after bilateral ureteral ligation, blood continues to flow through the organs, and the renal tissue remains viable for

2. Addis, T., *J. Lab. and Clin. Med.*, 1925, v10, 402.

† The difference between the observed means at the end of the 24th postoperative hour, or 22.7 mg per 100 cc of serum, was tested by Fisher's *t* test. The ratio of the difference to its sampling error was 22.7/9.64 = 2.35. Entering Fisher's table with 4 degrees of freedom, and halving the probability found, one finds a probability of approximately 4 in 100 that the difference obtained in the plus direction would occur by chance if the experiments were repeated. Furthermore, the observations made at the end of the 12th hour after ureteral ligation also fall to about the same degree below the nephrectomized level. For these reasons, the difference observed between the serum urea concentrations of the nephrectomized rats and of the animals subjected to bilateral ureteral ligation is considered to be statistically significant.

TABLE III.
In vitro Sensitivities of Aureomycin-Resistant Beta Hemolytic Streptococci After Transfer on Control Medium or Passage Through Mice.

Group	Strain	Parent, μg/ml	Resistant variant, μg/ml	Sensitivity after transfer on control medium, μg/ml				Sensitivity after 12 normal mouse passages, μg/ml
				25 × s	50	75	100	
A	S1	.5	1	1	1	1	1	
	S2	.5	3	3	3	3	1	1.8
	S3	.5	4	3	1	1	1	4.0
	S4	.5	3					3.0
B	T5	.08	1	1	1	1	1	1.5
	T7	.08	1					1.0
	T8	.08	2					2.0
	T10	.08	2	1	1	1	1	
	T11	.08	1	1	1	1	1	
C	U1	.1	1	1	1	1	1	0.5
	U2	1.0	60	50	7	7	7	3.0
	U3	1.0	20	30	3	3	3	8.0

most uniform in their rate of development of resistance. By group, the rate of development of resistance was A less than B less than C.

C. *Acquired Resistance on Control Medium.* All 23 parent organisms were subcultured serially on control medium (aureomycin-free blood agar). Dissociation was minimal and no changes in hemolysis were observed. After 40 transfers, no significant increase in aureomycin resistance was discernible.

D. *Loss of Acquired Resistance.* Three aureomycin-resistant strains of group A, 3 of group B, and 3 of group C streptococci were subcultured serially 100 times on aureomycin-free blood agar plates in an attempt to restore their original sensitivity. The organisms were tested on the 25th, 50th, 75th, and 100th transfer by the whole plate method. Only the 2 most resistant group C strains (U_2 and U_3) lost 5/6ths of the acquired resistance after the 50th passage on plain blood agar plates. Neither, however, returned to its original level of sensitivity. These values, recorded in Table III, remained unchanged over an 8-month period of storage in broth at 4°C. Three strains each of group A, B, and C streptococci after acquiring aureomycin resistance were passed intracerebrally 12 times in normal mice and their *in vitro* sensitivities determined by the whole plate technic. Only the 2 most resistant group C strains (U_2 and U_3) showed a significant decrease in resistance (Table III).

E. *Change in Virulence.* Only 3 strains of streptococci, the U_2 and U_3 group C and the S_2 group A strains, showed an appreciable loss of mouse virulence after acquiring aureomycin resistance. In general, the behavior of the control strains was similar to that of the parent organisms. In 5 of 6 group A, B, and C strains, the virulence of the resistant variant was increased over that of either the parent or resistant organism by 12 intracerebral passages in normal mice. These results are given in Table IV. The number of viable streptococci in cultures of the parent and resistant strains was essentially the same.

F. *Antigenic and Hemolytic Changes.* The 23 beta hemolytic streptococci were regrouped by the Lancefield technic after acquiring aureomycin resistance. In only 1 resistant group B organism, the T_5 strain, were we unable to demonstrate the group-specific precipitinogen. Transient conversion from beta to alpha hemolysis occurred on concentrations of aureomycin which limited growth in several group A, B, or C organisms. These strains, when growing on control medium, had the normal beta type of hemolysis. Two group C organisms which produced an area of greening beyond the clear zone, showed an exaggeration of this color when growing on low concentrations of aureomycin. This greenish hemolytic aura completely disappeared leaving only a clear beta zone as the inhibiting concentration of aureomycin was reached.

G. *Enzymatic Alterations.* Early results of comparisons of antigenic and enzymatic alterations produced in the same beta hemolytic streptococci by the separate induction of penicillin-, streptomycin-, bacitracin-, and aureomycin-resistance have been reported from this laboratory.(9) Aureomycin-resistant organisms, in general, showed a less altered metabolism, as measured by their streptolysin-S, proteinase, streptokinase and ribonuclease production, than did either penicillin- or streptomycin-resistant streptococci. No significant change in streptolysin S activity accompanied induced aureomycin resistance in 5 group A and 3 group C organisms surveyed. The 2 group A strains that produced proteinase showed unaltered enzyme activity after induced aureomycin resistance. However, reduced streptokinase production was demonstrated in 1 of 3 group A and 1 of 2 group C resistant variants when compared with their controls. Ribonuclease activity was lessened in 2 of 7 group A streptococci by transfer on aureomycin medium.

IN VIVO. A. *Acquired Resistance in Protected Mice or Embryonated Eggs.* The C203 MV strain of beta hemolytic streptococcus developed no *in vivo* aureomycin resistance after 25 serial mouse passages, nor was there a change in the *in vitro* sensitivity at the end of these passages. The PD_{50} for aureomycin and LD_{50} for the 1st and 25th passages respectively were essentially the same. The sensitivities of the parent, intermediate and final strains as determined by the whole plate technic were identical. Similarly, the C203 MV strain showed no acquisition of resistance to aureomycin through 30 egg passages, as measured by the successive amounts of aureomycin needed to sterilize the eggs and by the change in *in vitro* sensitivity of the passaged organism.

Discussion. Resistance to aureomycin may be induced in beta hemolytic streptococci by a technic similar to that used for penicillin or streptomycin. Many subcultivations are necessary on sub-inhibitory concentrations of the drug to produce a detectable change in the sensitivity of any of the streptococci employed. The magnitude and rate of the changes are quite comparable to those previously seen for penicillin.(3,5) Failure to demonstrate the development of aureomycin resistance *in vivo* in a group A strain was obtained also with penicillin.(6) Both the *in vivo* and *in vitro* results suggest that the development of aureomycin resistance in beta hemolytic streptococci will not be of clinical importance if an analogy between laboratory studies and clinical results for penicillin and aureomycin is valid. To date, penicillin resistance by beta hemolytic streptococci has not been of clinical importance. This had been predicted by several laboratory studies(11,12,3,6) on induced resistance. Aureomycin appears to produce alterations in the biology of streptococci less frequently than does streptomycin or penicillin. The mouse virulence is only infrequently decreased, the group antigenicity is unchanged, and the enzyme systems are only occasionally inhibited.

Summary. 1. The aureomycin sensitivity range of 274 recently isolated group A, B, and C beta hemolytic streptococci was 0.02-0.5 μg/ml.

2. Aureomycin resistance was induced in 23 strains of group A, B, and C beta hemolytic streptococci by 40 serial subcultivations on aureomycin medium. The minimum increase in resistance was 2-fold in a group A organism, and maximum increase was 60-fold in a group C organism.

3. Serial transfer on control medium or passage through normal mice partially restored aureomycin sensitivity to resistant variants.

4. Mouse virulence was decreased in only the 2 group C organisms which had shown the highest degree of aureomycin resistance.

5. In only 1 group B resistant variant was the group specificity lost after the development of aureomycin resistance.

6. Growth on maximum concentrations of aureomycin stimulated transient conversion from clear to green hemolysis in organisms normally producing only a clear beta zone. Conversely, group C organisms with normal

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TABLE IV.
Mouse Virulence of Group A, B, and C Beta Hemolytic Streptococci with Acquired Aureomycin Resistance.

Group	Strain	Initial LD_{50}		LD_{50} after 40 transfers on			LD_{50} of resistant strains after 12 mouse passages
				Control medium	Aureomycin medium		
		Dilution*	No. organisms†	Dilution*	Dilution*	No. organisms†	Dilution*
A	S2	$10^{-4.8}$	1.6×10^2	$10^{-2.8}$	$10^{-1.5}$	3.0×10^5	
	S3	$10^{-4.5}$	2.0×10^2	$10^{-3.7}$	$10^{-3.0}$	8.4×10^3	
	S4	$10^{-4.3}$	3.7×10^2	$10^{-3.2}$	$10^{-3.0}$	4.5×10^3	$10^{-6.2}$
B	T5	$10^{-3.0}$	1.9×10^4	$10^{-4.8}$	$10^{-2.5}$	4.3×10^4	$10^{-7.0}$
	T7	$10^{-4.0}$	6.3×10^2	$10^{-5.3}$	$10^{-4.0}$	5.9×10^2	$10^{-3.2}$
	T8	$10^{-4.0}$	7.8×10^2	$10^{-6.3}$	$10^{-4.5}$	8.5×10^2	$10^{-7.0}$
C	U1	$10^{-1.5}$	2.1×10^5	$10^{-2.5}$	$10^{-1.3}$	1.0×10^6	$10^{-3.6}$
	U2	$10^{-6.0}$	9.3×10^1	$10^{-4.0}$	10^{-0}	2.2×10^7	
	U3	$10^{-2.5}$	5.8×10^4	$10^{-2.8}$	$10^{-1.2}$	2.0×10^6	$10^{-5.7}$

* The LD_{50} is expressed in terms of the dilution of the whole broth culture after it had been adjusted in density to that of its corresponding parent strain.
† The LD_{50} is expressed in terms of the number of viable streptococci inoculated.

green hemolytic characteristics lost their green hemolytic zones when grown on maximum aureomycin concentrations.

8. Reduction in streptokinase production occurred in 2 of 5 group A and C resistant strains and diminished ribonuclease activity in 2 of 7 group A resistant variants. No change in streptolysin S or proteinase activity was observed.

9. The C203 MV strain of group A streptococcus failed to acquire aureomycin resistance after repeated exposures to sublethal amounts of the antibiotic either in mice or embryonated hens' eggs.

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VII. Vitamin B_{12} and the Intrinsic Factor. (17560)

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Soon after the discovery by Minot and Murphy(1) of the effectiveness of liver in the dietary treatment of pernicious anemia, it was shown that foods such as beef muscle contain a heat-stable substance, designated the extrinsic factor,(2) which produces little or no response when ingested by a pernicious anemia patient unless normal gastric juice is also administered with it. The activity of gastric juice has been ascribed to the so-called intrinsic factor; gastric juice alone has no oral hematopoietic power.(3) Over a period of years the relationship between the extrinsic and intrinsic factors has been the subject of considerable clinical experimentation by Castle and his associates, and other investigators. Recent findings on this subject are as follows: Vitamin B_{12}, active parenterally at the microgram level, has been found to be essentially inactive when administered orally

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