pared with the result obtained following the administration of the acid hydrolysate to which tryptophane and cystine had been added. This difference in action between the two types of casein hydrolysate used in this experiment may be explained on the basis that in the acid-base hydrolysate almost half of the amino acids administered were in the racemic form due to the procedure employed in hydrolyzing 50% of the casein with barium hydroxide.

Summary. A method for the preparation of a casein hydrolysate suitable for parenteral administration is described. Data are presented showing greater utilization and deaminization following the parenteral administration into fasting rabbits of an acid hydrolysate of casein to which l-tryptophane and l-cystine had been added, than following the administration of either glycine or partially racemized amino acids of an acid-base hydrolysate of casein.

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Enhancing Effect of Nicotinic Acid and Cysteine Hydrochloride on Growth of *Leptospira icterohaemorrhagiae*.*

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While carrying out various studies on *L. icterohaemorrhagiae*, the difficulty of growing these organisms in quantities suitable for use as antigen in the agglutination or complement fixation tests constantly presented itself. Likewise, some difficulty had been encountered in perpetuating strains of leptospira in culture media, since they died for no apparent reason, and batches of media prepared identical to previous ones would often not sustain the growth of these organisms.

In an attempt partially to resolve these difficulties, the effect of the addition of various amino acids and nicotinic acid to a modified Noguchi semi-solid medium was tested.

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Experimental Methods. The basic medium used in these studies was prepared as follows: To a mixture of 10 cc normal rabbit serum, 1.5 cc hemoglobin (1 part rabbits' blood to 3 parts sterile distilled water) and 68.5 cc normal saline was added 10 cc of a previously melted 2% infusion agar. To this basic medium was later added 10 parts of the solution containing the growth factor to be tested.

The solutions to be added were prepared by dissolving 50 mg of nicotinic acid or one of the amino acids in 50 cc of normal saline. The solution was sterilized by filtration through a Seitz pad. Eight solutions, containing, respectively, nicotinic acid, cysteine hydrochloride, tyrosine, valine, glutamine, leucine, glycine, and methionine were prepared, and 10 cc of each added to 90 cc of the basic medium described above. In one tube, which served as the control, 10 cc of normal saline were added to the basic medium. In each experiment, the basic medium was prepared originally in a single batch.

A few observations were also made on the effect of a saline extract of yeast when added to the basic medium in a final concentration of 0.2%. The yeast solution was made by dissolving 2 g of Difco yeast in 100 cc of normal saline and filtering the solution through a Seitz pad.

The different media were then tubed in 15 cc amounts, and heated at 57°C for 30 minutes. Subsequently, the tubes were inoculated with 0.5 cc of an actively growing culture of *L. icterohaemorrhagiae* in Noguchi's medium to which no growth factor had been added. In each experiment 5 or 6 different tubes of each medium were tested. Four separate experiments were run, although in one experiment cysteine and leucine were omitted. All tubes were incubated at 32°C, and were examined daily by the darkfield microscope.

Estimation of the growth in the tubes on a given day was based on a rough count of leptospira seen under the darkfield in 50 fields of a slide prepared from each tube. The approximate degree of growth was recorded as follows: No leptospira seen, —; 1-10 leptospira in all 50 fields, ±; 10-50 organisms in a total of 50 fields, +; an average of 3-20 leptospira per field, ++; 20-100 spirochetes per microscopic field, +++; leptospira in each field too numerous to count, ++++. All tubes designated as +++ or ++++ were considered to be satisfactory for use as antigen for the agglutination or complement fixation tests.

Results. In Table I are given the composite results of 4 different experiments. Each reading noted, with the exception of

those for cysteine and leucine, is based on at least 20 tubes of a medium containing one added substance. The results show that the addition of nicotinic acid in a final concentration of 0.01% (10,000 gamma per 100 cc) materially accelerated the growth of leptospira, as compared with the standard media, so that only 3 or 4 days were required to obtain a maximum yield. Cysteine hydrochloride also accelerated the growth, though not as much as nicotinic acid. The results admittedly are based on rough estimates of the number of leptospira in each tube, but the differences were definite and consistent.

The tubes of media to which had been added glycine always showed poor growth, and few viable leptospira were demonstrable after 6 days' incubation at 32°C. The addition of valine, tyrosine, methionine, glutamine, and leucine seemed to have little enhancing effect on the growth of leptospira, and there were no marked differences between these tubes and the control tubes.

The effect of yeast, in the few observations made, was enhancing, though not as much so as nicotinic acid and cysteine. Zimmermann[1] also observed that the addition of a loopful of sterile culture of yeast to 1.0 cc of ordinary medium enhanced the growth of leptospira.

Summary. Nicotinic acid enhances the growth of leptospira when added to Noguchi semi-solid medium in a final concentration of 0.01%. Cysteine hydrochloride in the same concentration has a similar effect, though not as marked as nicotinic acid. Glycine seems to have a reverse effect, and may even act to suppress the growth of the organisms.

TABLE I.

Effect of Nicotinic Acid and Certain Amino Acids on Growth of Leptospira in Modified Noguchi Medium.

Substance added to basic medium	Degree of positivity* Days after inoculation						
	1	2	3	4	5	6	7
Nicotinic acid	+	++	+++	++++	++++	++++	++++
Cysteine hydrochloride*	±	+	+++	+++	++++	++++	++++
Tyrosine	—	±	+	++	+++	++++	++++
Valine	±	+	+	++	+++	++++	++++
Glutamine	±	+	++	+++	++++	++++	++++
Leucine*	±	+	++	+++	+++	++++	++++
Glycine	±	±	±	±	±	±	±
Methionine	±	+	++	+++	+++	++++	++++
Control	±	+	++	+++	+++	++++	++++

*Based on 3 experiments with a total of 18 tubes.

1 Zimmerman, E., *Zent. f. Bakt.*, 1936, **137**, 280. (Abstract—*Trop. Dis. Bull.*, 1937, **34**, 358.)