mechanically ground with carborundum (grit 600) in a glass tube and pestle. The supernatant showed no differences with and without added antibody. Furthermore, a paste of organisms was frozen at −78°C and placed in a cylinder at the same temperature; a piston was struck with a heavy weight in order to disintegrate the bacteria. Smears showed very few intact organisms remaining after such treatment. The supernatant of this material as well as the disintegrated residue itself was inactive.

Although certain hypotheses come to mind, at the present time we do not feel a discussion of this phenomenon is warranted. It should be pointed out that experiments heretofore reported have ordinarily employed 24-hours cultures; no investigations have been previously carried out using organisms during the early stages of their growth. Experiments are now in progress with other organisms. In addition synthetic media of known composition are being investigated in an attempt to elucidate this phenomenon more fully.

Summary. When growing bacteria, *S. marcescens*, are placed in contact with their specific antibody and with normal serum or gamma globulin there is an increased O_2 consumption by the former over the latter, particularly when CO_2 is absorbed.

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Studies on the Nutrition of *Leptospira canicola.** (18147)

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The metabolism of various amino acids, carbohydrates, vitamins and salts utilized by pathogenic leptospira has been investigated by a number of workers(1-8) but there has been no report of a chemically-defined medium adequate to support growth of this group of organisms.§ Chang(1) and others(2,4,9) have shown that serum is a necessary constituent of the growth medium for many of the leptospira strains, and the presence of this complex substance in test media has considerably limited nutritional studies with these microorganisms. It was the object of the present investigations, therefore, to develop a semisynthetic medium for *Leptospira canicola* which would permit the determination of some of the vitamin and amino acid requirements of this species.

Experimental. The media were based on

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§ Savino and Rennella(5) have reported success in maintaining a *Leptospira icterohemorrhagiae* strain, but not other strains, in a chemically-defined medium consisting of minerals, hematin, asparagine, dextrin and activators (nicotinic acid, nicotinamide, thiamine, pyridoxine, riboflavin, aspartic acid and pimelic acid).

9. Stuart, R. D., *J. Path. and Bact.*, 1946, v58, 343.

Schüffner's medium described by Greene(2) and Rosenfeld and Greene(4). The basal medium was prepared by dissolving the following salts in water and autoclaving and filtering the resulting solution (concentrations given in mg %): Na_2HPO_4 (116), KH_2PO_4 (154), NaCl (468), Na_2CO_3 (11.7), $CaCl_2$ (11.7), and KCl (11.7). 1.6 ml of the basal medium and enough water to make a final volume (including test samples and inoculum) of 7.0 ml were added to each 15 x 127 mm test tube. Heat-labile materials were filter-sterilized and added aseptically after sterilization of the tubes. Heat-stable materials were added before sterilization. The test-tubes were plugged with cotton and sterilized in the autoclave for 30 minutes at 15 lb.

Leptospira canicola‖ was carried by weekly transfer according to the following procedure. The culture medium (pH 7.2-7.4) was prepared by placing 1.6 ml of basal medium and 4.0 ml of water with 4.68 mg of Witte's peptone in each test-tube. After autoclaving the tubes 0.7 ml of filter-sterilized rabbit serum¶ and 0.7 ml of a 7-day culture of *Leptospira canicola* were added aseptically to each tube. The tubes were incubated for 7 days at 32°. A fresh 7-day culture was centrifuged and resuspended in a sterile mixture containing 5.4 ml of water and 1.6 ml of basal medium to prepare an inoculum suspension for use in the nutrition experiments. 0.4 ml or 0.7 ml of freshly prepared inoculum suspension was used to inoculate each tube. After 7 days incubation at 32° the growth in each tube was estimated by measuring the turbidity of the culture with the aid of the Klett-Summerson photoelectric colorimeter. In some of the experiments the turbidity results were verified by counting the leptospira in the cultures with the aid of the dark-field microscope.

Serum fractions. The basal medium was supplemented with 4.68 mg of Witte's peptone per tube in experiments designed to determine the relative activities of serum and serum fractions in promoting growth of *Leptospira canicola.* It was found that rather extensive dialysis** of the rabbit serum did not appreciably reduce its activity. The response of *Leptospira canicola* to dialyzed rabbit serum in a number of experiments is given in Table I.

Treatment of the dialysed serum with trypsin at pH 8.0 did not reduce the protein content†† or the growth-promoting activity of the preparation. It was assumed that the native proteins of the serum were refractory to trypsin since heated preparations were readily digested. The heated preparations were inactive in promoting growth both before and after digestion with trypsin. Treatment of the dialysed serum with pepsin at pH 3.0 resulted in digestion of the protein and inactivation of the preparation for the test organism. Activity was retained in control samples of dialysed serum held at pH 3.0 under the same conditions. Since the samples were filter-sterilized before testing, it is apparent that the pepsin may have eliminated the essential serum factor either by digesting it or by making it insoluble (a precipitate formed in the pepsin-treated preparation). Fractionation of the dialysed serum by ammonium sulfate precipitation at room temperature followed by dialysis of the fractions to remove the ammonium sulfate resulted in a loss of about 80% of the original activity of the dialysed serum as determined by testing the growth-promoting activity of the combined fractions. The remaining activity did not appear to be concentrated in any one of the fractions which were obtained. It

‖ Strain obtained from Dr. K. F. Meyer, University of California, San Francisco.

¶ Pooled blood from decapitated rabbits was stored overnight in a cold room, the serum (containing hemoglobin from partial laking of the red cells) was sterilized by filtration and the sterile filtrate was stored in the refrigerator. The authors are indebted to Mr. Boone of Fontana, California for the rabbits, to Mr. E. A. Murphy and Dr. W. Drell for assistance in collecting the blood, and to the Hyland Laboratories (Los Angeles) for filtering and bottling the serum.

** 100 ml aliquots of serum were placed in cellulose sausage casings (The Visking Corporation, size 36/32) and suspended in 5 gallon bottles of distilled water in the refrigerator. The distilled water was frequently changed over a 10-day period, and the resulting dialysed serum was filter-sterilized, and kept in sterile bottles in the refrigerator.

†† Tested by precipitation with trichloracetic acid.

TABLE I. Response of *Leptospira canicola* to Dialysed Rabbit Serum.

Dialysed rabbit serum, ml/tube	Increase in photometer readings after incubation								
	Preparation 1			Preparation 2	Preparation 3		Preparation 4		
	Trial 1	Trial 2	Trial 3		Trial 1	Trial 2	Trial 1	Trial 2	Avg
.14	10	2	3	9	4	6	11	9	6.6
.28	18	9	15	15	12	13	18	14	14
.42	24	22	14	19	21	16	30	23	21
.56	33	26	34	33	30	26	40	28	31
.70	36	39	39	32	37	33	46	36	37

The ml of dialysed serum per tube was calculated as ml of serum before dialysis. Preparations 1 to 4 of dialysed serum were prepared 10/23/48, 7/6/48, 11/12/48, and 12/3/48, respectively. The original serum was collected in March, 1948.

TABLE II. Response of *Leptospira canicola* to Witte's Peptone and to Amino Acid and Vitamin Mixtures.

Additions to media	Increase in photometer readings after incubation					
	Basal medium without peptone			Basal medium with peptone		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
None	15(14-16)	12(11-13)	11(10-11)	32(32-32)	28(28-28)	28(26-30)
Amino acid mixture*	25(24-26)	21(19-25)	26(25-27)	35(34-36)	34(31-37)	36(33-37)
Amino acid + vit. mixture†	32(31-34)	31(29-33)	30(30-31)	37(35-38)	35(33-39)	37(35-39)

The given values are the averages of 2 determinations in the media with no additions and of 5 or 6 determinations in the others. All media contained dialysed serum as described in text. The ranges of the determinations are given in the parentheses.

* Solution A (described in text) plus glutamine.

† Solutions A and B (described in text) plus glutamine.

seemed likely from the experiments just described that the active factor in the serum was a protein. The loss in activity resulting from ammonium sulfate precipitation was probably due to denaturation of the essential protein.

It is of interest that both the dialysable and non-dialysable fractions of bovine serum contained substances active in maintaining the Reiter strain of *Treponema pallidum* according to the reports of Little and SubbaRow (10) and Whitely and Frazier(11). These investigators have found also that crystalline bovine serum albumin is active for this strain and this finding has been confirmed by Eagle and Steinman(12). Crystalline human and bovine plasma albumins‡‡ had little activity in replacing the dialysed rabbit serum for *Leptospira canicola* in the present investigation.

Peptone requirement. In the preceding experiments it was found that the basal medium supplemented with Witte's peptone and dialysed rabbit serum supported adequate growth of *Leptospira canicola* (Table I). When Witte's peptone was omitted whole serum, but not dialysed serum (Table II), supported adequate growth. It was apparent, therefore, that essential substances removed from serum by dialysis were supplied by Witte's peptone. The basal medium was supplemented with dialysed serum (in amounts equivalent to 0.7 ml of rabbit serum per tube) in experiments to determine the peptone requirement of *Leptospira canicola*. Trypsin digested casein was found to substitute adequately for Witte's peptone, and in subsequent experiments, 1 ml of the following

10. Little, P. A., and SubbaRow, Y., *J. Immunol.*, 1945, v56, 213.

11. Whiteley, H. R., and Frazier, C. N., *Am. J. Syph., Gonor. and Ven. Dis.*, 1948, v32, 43.

12. Eagle, H., and Steinman, H. G., *J. Bact.*, 1948, v56, 163.

‡‡ The crystalline bovine plasma albumin was a product of Armour Laboratories, Chicago, Ill. The authors are indebted to Dr. E. J. Cohn for a gift of the crystalline human plasma albumin.

amino acid solution per tube, supplemented with 0.7 mg of L-glutamine (heat labile) per tube, was found to substitute partially (Table II) for the peptone (concentrations given in mg %): DL-alanine (51.9), L-arginine monohydrochloride (51.9), L-asparagine monohydrate (5.18), L-cysteine hydrochloride (10.4), glycine (5.18), L-histidine monohydrochloride monohydrate (5.18), DL-isoleucine (51.9), L-leucine (26.0), DL-lysine monohydrochloride (10.4), DL-methionine (5.18), DL-norleucine (10.4), DL-norvaline (10.4), DL-phenylalanine (26.0), L-proline (5.18), DL-serine (10.4), DL-threonine (10.4), DL-tryptophan (5.18), L-tyrosine (10.4), and DL-valine (38.9). The solution just described will be referred to hereafter as Solution A, and unless otherwise indicated the amount used per tube was 1.0 ml.

Growth equivalent to that obtained with peptone (Table II) was obtained with the amino acid mixture (Solution A) when it was further supplemented with 0.7 ml of the following vitamin-purine-pyrimidine-salt solution per tube (concentrations given in micrograms per cent): thiamine hydrochloride (6.7), pyridoxine hydrochloride (10.7), pyridoxal hydrochloride (0.67), pyridoxamine dihydrochloride (0.67), calcium DL-pantothenate (13.4), riboflavin (13.4), niacinamide (13.4), biotin (0.034), *p*-aminobenzoic acid (0.67), folic acid (0.034), choline chloride (51.1), inositol (168), adenine sulfate (80), guanine (80), uracil (80), xanthine (80), $MgSO_4$ (658), $FeSO_4 \cdot 7H_2O$ (67), and $MnSO_4 \cdot H_2O$ (51.1). The solution just described will be referred to hereafter as Solution B, and unless otherwise indicated the amount used per tube was 0.7 ml.

The basal medium supplemented with dialysed rabbit serum and Witte's peptone was found to support growth of *Leptospira canicola* through a prolonged series of transfers. When the peptone was replaced by Solutions A and B plus glutamine good growth was obtained in the first transfer, but growth was reduced in subsequent transfers. It was apparent, nevertheless, that the semisynthetic medium consisting of the basal medium supplemented with dialysed rabbit serum, glutamine, and Solutions A and B might serve as a basis for determining many of the amino acid and vitamin requirements of *Leptospira canicola*. Experiments to determine these requirements as well as further studies on the nature of the serum factor and the peptone factors required for good growth in serial subcultures are in progress. The results of these experiments are to be given in subsequent papers from the authors' laboratories.

Summary. Nutritional experiments with *Leptospira canicola* have been described. A semisynthetic medium containing dialysed rabbit serum, salts, vitamins, amino acids, and purine and pyrimidine bases was developed and appeared to be adequate as a basis for determining amino acid and vitamin requirements of this organism.

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Respiration of Embryonic Blood. (18148)

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Embryonic respiration has long been a matter of great interest to physiologists. A review of extensive literature on the subject will not be attempted. Hall(1) briefly reviews contemporary works on the respiratory role of the blood in his report on hemoglobin function in the blood of the chick embryo. These works, however, have been largely qualitative and concerned with the changes in the character and function of hemoglobin

1. Hall, F. G., *J. Physiol.*, 1934, v83, 222.