30 mg injection of cortisone was approximately one week. This prolonged effect was probably due, in part at least, to the insolubility of the compound and its resultant slow absorption, because upon examination the material could often be seen at the site of injection 2 or 3 days and sometimes longer after it had been given.

The failure of ACTH to affect anaphylactic shock in guinea pigs confirms the earlier observations of Leger, Leith and Rose. Employing a larger dose of ACTH in the present study and testing at various intervals after the administration of the hormone did not affect the results. Wolfram and Zwemer(4) noted a brief period from 2½ to 5½ hours after the administration of adrenocortical extract during which sensitized guinea pigs were apparently partially protected against injections of the sensitizing agent. In the present study tests for sensitivity were not made during this early period.

Summary. Guinea pigs were used throughout this study. The number of eosinophils in the blood of males was significantly lower than that in females. Cortisone reduced the number of eosinophils in the blood of males and females. ACTH produced a pronounced eosinopenia in males and nonpregnant females. Pregnancy abolished the eosinopenic effect of ACTH. Neither cortisone nor ACTH had any effect upon the degree of anaphylactic shock produced in guinea pigs by the intravenous injection of the agent to which the animals had been sensitized.

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Effect of Specific Antibody on the Metabolism of *Serratia marcescens.** (18146)

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From time to time studies have been carried out to determine any possible effect which specific antisera might have on the growth and metabolism of various bacteria. We shall not attempt at this time to survey the literature on this subject which has been adequately covered by Oldfelt(1) and Sevag (2). Suffice it to say, with few exceptions, there is agreement that when a bacterium comes in contact with its specific antiserum little or no change can be demonstrated in its metabolism as measured by oxygen consumption, providing lysis of the organism does not occur. Because of some preliminary experiments we have felt that this problem should be re-studied, using simple substrates, purified (ethanol precipitated) antibody and suspensions of resting (washed) organisms.

Experimental. Rabbits were injected intravenously 6 times at 2- or 3-day intervals with emulsions of live *S. marcescens* prepared from cultures grown for 24 hours on pancreatic digest agar. Twelve days after the last injection the animals were bled from the heart. Each serum was inactivated at 60°C for 3 minutes and was then titrated against live organisms. After finding the sera to be active (titer: 1-20,480 to 1-40,960) all were pooled. A portion of this was frozen and used as necessary. The remainder was treated with ethanol to obtain the gamma globulin fraction as described by Cohn *et al.*(3). Normal rabbits were bled; after inactivation

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some of the serum was frozen; the rest was treated with ethanol.

To obtain suspensions of resting organisms, 9-hour cultures grown on trypticase-soy† agar were washed from the medium with phosphate buffer (pH 7.4). They were centrifugalized, the supernatants decanted, new buffer added and the tubes recentrifugalized. This process was repeated 3 times. Three cc of buffered Krebs-Ringer solution containing the substrate were placed in the main chamber of each Barcroft-Warburg reaction vessel together with .1 cc of an appropriate suspension of organisms. The concentration of bacilli giving optimum O_2 consumption had to be determined by trial and error; after this was obtained it was standardized against a suspension of $BaSO_4$ so that density of organisms could be repeated at will. One-tenth cc of antiserum and normal serum or various concentrations of precipitated antibody and normal gamma globulin were placed in the arms of respective flasks. Two-tenths cc 10% KOH were placed with filter paper in the center cups. Observations were made for periods up to 6 hours. A variety of substrates were studied: glucose, succinate, malate, mannite, sorbitol, inulin. With all of these substrates no differences in O_2 uptake could be found between those flasks containing antibody and those containing normal serum.

Before abandoning the experiment it was decided to study the effect, if any, of antibody on the growing organisms. Accordingly, an 8-hour culture grown in pancreatic digest broth was diluted 1-500 with trypticase soy broth; 3 cc of this were placed in the main chamber of reaction vessels. Antibody or normal serum were added to respective side arms, while 10% KOH was placed in the center wells. With this dilution of organisms there was very little uptake of O_2 during the first 2 hours. By the third hour it was apparent that the flasks containing antibody were showing an increased O_2 uptake and the difference became even more marked during the ensuing 2 hours. In the following hours as the concentration of organisms in each group of flasks increased the oxygen uptake became too large to measure. A typical experiment is shown graphically in Fig. 1. These results have been uniformly consistent.

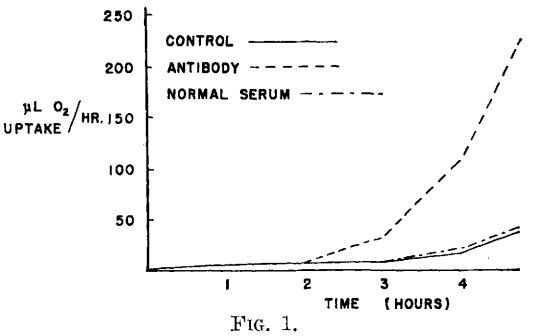

FIG. 1.

Oxygen uptake in micro liters per hour at varying times. Control flask contained medium and bacteria; normal serum flask contained medium, bacteria and .1 cc normal rabbit serum; antibody flask contained medium, bacteria and .1 cc rabbit antiserum for *S. marcescens*.

The difference in rate of O_2 uptake may be increased if 100% O_2 rather than 20% O_2 makes up the gas phase in the flask. If CO_2 is not absorbed the difference is negligible. There is a more rapid fall in pH in the medium containing antibody; this difference, though not large (0.2 pH in 4 hours) has been consistent. In addition, an increased glycolysis as evidenced by a fall in concentration of reducing substances in the media containing antibody has been noted. We have not been able to detect any difference in O_2 uptake when washed organisms have been utilized with trypticase-soy broth as substrate. This, we feel would seem to indicate that growing organisms are necessary to elicit the phenomenon. Furthermore, no differences have been elicited when growth has been inhibited by appropriate concentrations of streptomycin.

Because of the possibility that lysis might be taking place and might account for the phenomenon we have deliberately added guinea pig complement to the system, since the antiserum had been inactivated. This had no effect. Washed organisms were suspended in water at 37°C in an attempt to produce autolysis; after 24 hours the supernatant fluid was tested against the medium. There was no O_2 uptake whatsoever. In addition, a heavy suspension of organisms was

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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).

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