TABLE IV. Response of *Leptospira canicola* to Different Serum Albumins.

Albumin	Activity**
1. Crystalline bovine, No. 1*	0
2. human†	0
3. Control (dialysed rabbit serum)	36
4. Crystalline bovine, No. 2‡	10
5. sheep‡	12
6. Salt-precipitated sheep§	30
7. bovine§	9
8. horse	45
9. rabbit§¶	24
10. Control (dialysed rabbit serum)	33

* Alcohol-fractionated product obtained from Armour Laboratories, Chicago, Ill.

† Alcohol fractionated product obtained through the courtesy of Dr. E. J. Cohn, Harvard University.

‡ Alcohol fractionated product obtained through the courtesy of Lederle Laboratories, Pearl River, N. Y.

§ Prepared by the authors from whole blood obtained from the Los Angeles Wassermann Supply Co., Los Angeles.

Prepared by the authors from whole blood obtained from the Victory Slaughtering Co., Los Angeles.

¶ 1 ml (4.98 mg N/ml) used per tube.

** Increase in optical density (each value average of 5 determinations) after 7-days incubation at 32°. The chemically-defined basal medium was used with samples 1-3 and the stock medium supplemented with peptone and minerals with samples 3-8.

in Table IV. Generally, the crystalline and the alcohol-precipitated albumins lacked, or had low, activity while the salt-precipitated albumin fractions (except bovine) exhibited activity nearly comparable to that of the control (dialysed rabbit serum). Savino and Rennella(2) reported that hematin was a growth factor for *L. icterohemorrhagiae* but it seems unlikely that the growth effects observed in the present experiments are accounted for by heme compounds. It may be noted that traces of heme compounds were present in the inactive, as well as the active, fractions of the rabbit and horse sera.

In further experiments it was found that salt-precipitated horse or sheep albumin could substitute satisfactorily for dialysed rabbit serum or salt-precipitated rabbit albumin in supplementing a basal medium containing only peptone and salts. Subculturing of *L. canicola* was not attempted although it was determined that *L. icterohemorrhagiae* could be progagated satisfactorily by transferring it weekly over a 3-month period in the peptone-salts-rabbit serum albumin medium. It is of interest that Chang(3) was unable to maintain *L. icterohemorrhagiae* in a medium in which horse serum was replaced by crystalline bovine serum albumin.

Summary. *Leptospira canicola* has been cultivated satisfactorily on a chemically-defined medium supplemented with electrophoretically-homogeneous rabbit albumin separated from serum by ammonium sulfate fractionation. The results of experiments with crystalline bovine and human serum albumins and with salt-precipitated albumin fractions from bovine, horse and sheep sera have been presented.

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Received November 26, 1951. P.S.E.B.M., 1951, v78.

Rate of Potassium Exchange in the Rat Erythrocyte.* (19216)

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Previous studies have shown that the human erythrocyte utilizes nearly a millimole of glucose for every millimole of potassium that enters the cell(1). The following experiments were undertaken to determine if a similar relationship existed in the metabolism of the rat erythrocyte.

Mature white rats of the Wistar strain were

* This work was supported in part by the U. S. Atomic Energy Commission.

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killed by a blow on the head and the abdomen and chest were immediately opened. Blood from the heart or vena cava was drawn into a syringe containing heparin. Using the methods previously described(1), the blood was mixed with an isotonic medium containing inorganic salts and glucose. The final percentage of cells of the diluted whole blood was between 10 and 20. $K^{42}Cl$ was incorporated into the diluting medium as a tracer. The diluted whole blood was incubated at 35°C to 36°C in rocker-dilution vessels(2) and equilibrated with 5% carbon dioxide and 95% oxygen. Four or 5 samples were withdrawn periodically during the course of the 10 to 12 hour incubation period for determinations of the percentage of cells (Hct), the diluted whole blood potassium and radioactivity, the diluted plasma potassium ($[K^+]p$), and the diluted plasma radioactivity ($K^{42}p$). Glucose determinations on the diluted whole blood were also done at the beginning and end of the experiment.

As some leakage of the intracellular potassium occurred during the course of the experiments, the rate of entrance of potassium into the rat erythrocytes from the suspending medium was determined by plotting the log of the radioactivity of the diluted plasma compartment, $K^{42}p$ x (1-Hct), against time. The initial slope of the first hourly period was used to calculate the per cent of the radioactivity of the diluted plasma compartment entering the cells per hour. The number of millimoles of potassium exchanging per hour was calculated from this percentage by multiplying it by the number of millimoles of potassium present in the plasma compartment, $[K^+]p$ x (1-Hct). As determined in 8 experiments, the mean value for the number of millimoles of potassium exchanging per hour per liter of cells was 5.24. The mean value for the percentage of the erythrocyte potassium which was exchanging every hour per liter of cells was 4.99. Glucose was utilized at the rate of 2.47 millimoles per liter of rat red cells per hour. A summary of the data from these experiments is shown in Table I.

TABLE I. Amount of Potassium Exchanged and Glucose Utilized by Rat Erythrocytes.

Exp. No.	% of cell K exchanging per hr	mM of cell K exchanging per hr per L of cells	mM glucose utilized per hr per L of cells
1	4.10	3.50	—
2	5.97	6.35	—
3	4.70	5.06	2.17
4	4.53	5.18	1.89
5	5.26	5.47	2.67
6	5.78	5.94	2.89
7	4.81	5.23	2.72
8	4.77	5.21	2.50
Mean	4.99	5.24	2.47

The number of millimoles of potassium exchanging per liter of red cells per hour for the rat is about 3½ times greater than that found for the human(1). On a cell surface area basis, the rat cell exchanges nearly three times as much potassium per unit time as does the human erythrocyte. These comparisons are shown in Table II.

Summary. (1) 5.0% of the intracellular potassium of the rat erythrocyte exchanges every hour, or 5.2 millimoles of potassium per liter of cells per hour. (2) 2.5 millimoles of glucose are utilized per hour per liter of rat erythrocytes. (3) The rat erythrocyte exchanges about 3½ times as much potassium per unit time as does the human erythrocyte. (4) The human erythrocyte utilizes nearly a millimole of glucose for every millimole of potassium that enters the cell. The rat erythrocyte utilizes less than ½ a millimole of

TABLE II. Comparison of Potassium Exchange and Glucose Utilization of Human and Rat Erythrocytes.

	mM K exchanging per L red cells per hr	mM K exchanging per μ^2 surface area per hr*	mM glucose utilized per L red cells per hr	mM glucose utilized per μ^2 surface area per hr*
Human erythrocyte	1.52	1.14×10^{-15}	1.39	1.05×10^{-15}
Rat "	5.24	3.26×10^{-15}	2.47	1.52×10^{-15}

* Calculated on the basis of the surface area of a human erythrocyte being 119 square microns and of the rat erythrocyte being 86 square μ (3).

glucose for every millimole of potassium that enters the cell.

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Received October 29, 1951. P.S.E.B.M., 1951, v78.

The Hemolytic Action of Gramicidin and Tyrocidin. (19217)

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Tyrothricin, an antibiotic complex isolated by Dubos(1) from the soil organism *Bacillus brevis*, consists of at least 2 hemolytic substances in the following approximate proportions: tyrocidin, 85%, and gramicidin, 15%. The hemolytic activities of these 2 substances as well as that of tyrothricin have been reported by various research workers(2-6). Quantitative data on the rates of reaction of the pure substances as well as mixtures are lacking, however. This paper presents comparative data on the hemolytic activities of gramicidin and tyrocidin and various mixtures. The analyses were made under the conditions developed by the author(7) for quantitative determination of tyrothricin.

Materials and methods. Stock alcohol solutions of the 2 purified substances† were made to contain 20 μg per ml of gramicidin or tyrocidin. Working solutions and mixtures were prepared by dilution with 95% alcohol.

One-half ml aliquots of the diluted solutions were added to 10 ml of a 0.25% twice-washed rat erythrocyte suspension in 0.87% saline, and the course of hemolysis was determined with a Klett-Summerson photoelectric colorimeter. A 660-millimicron filter was employed. Scale readings are proportional to optical density.

Action of gramicidin and tyrocidin. The course of hemolysis is markedly different for these two agents (Fig. 1). The hemolytic effect of gramicidin is represented by a sigmoidal curve with a pronounced lag period. The length of this period is dependent upon the concentration of gramicidin and the temperature. Tyrocidin, on the other hand, causes very rapid hemolysis, which is inhibited after about 10 minutes. The extent of hemolysis is dependent upon the concentration of the tyrocidin. Experiments discussed below indicate that the tyrocidin combines with some component of the cell stromata and becomes inactivated.

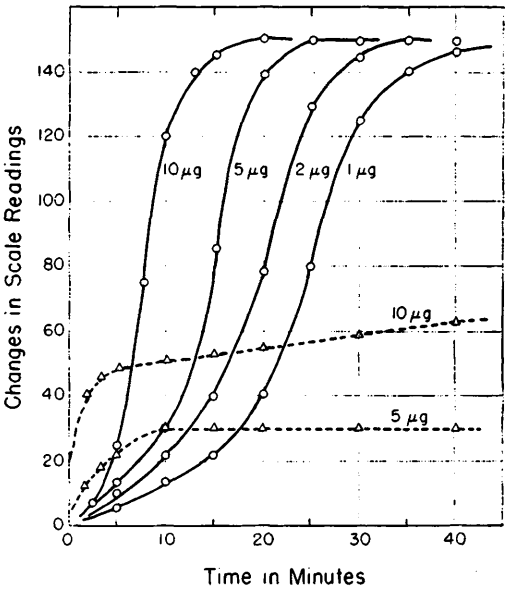

FIG. 1. Hemolysis curves for gramicidin and tyrocidin. Solid lines, gramicidin; broken lines, tyrocidin.

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† Several times recrystallized gramicidin and tyrocidin hydrochloride were prepared from crude tyrothricin according to methods of Hotchkiss and Dubos(4).