precipitate was found in the rest of the tubular system.

The method has also been used in the study of the renal injury produced by intravascular hemolysis and by the injection of solutions of hemoglobin. These results will be reported in detail subsequently.

Summary. Gersh's ferrocyanide histochemical renal function technic, slightly modified, has been found useful in studying experimentally produced renal lesions. In contrast to the finding of Gersh that such preparations fade rapidly, these have shown no change after 9 months.

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A Dialyzable Medium for Cultivation of Group A Hemolytic Streptococci.*

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In the course of work with the filtrates of streptococcal cultures a need was felt for a purified medium capable of supporting abundant growth of the organisms. Synthetic media of the present day did not appear to meet the requirements fully since no one of these media is uniformly capable of supporting heavy growth of all pathogenic strains of interest. Ultimately such defined media should be sufficiently developed to supplant the procedures described in this report.

Since it had previously been shown by Stock[1] that some toxin-producing streptococci are capable of growing on a medium containing only dialyzable constituents, a trial was made with heart muscle extract and commercial peptone purified by preliminary dialysis. It was found that the medium made with these purified constituents actually allowed more rapid and abundant growth in most cases than the standard Todd-Hewitt broth[2] of similar composition. The success of these efforts suggested that the medium might find a wider use than had originally been contemplated.

Preparation of the Purified Beef Heart Extract. Beef heart, after being stripped of fat and finely ground, is soaked overnight at 4°C in water,† 250 cc per pound of meat. Following this infusion the mixture is heated to 85°C for 30 minutes. This amount of heating appears to be just sufficient to coagulate muscle proteins and to express intracellular fluid, yet sufficiently mild to spare most of the heat-labile growth factors. A ten-fold reduction in the quantity of meat required is made possible by this minimal exposure to heat; with more violent extraction or with subsequent heat sterilization of the completed medium, a larger quantity of meat is needed

At the end of the 30-minute heating period, the extract is filtered through fluted filter paper (supported under the point of the cone with cheese-cloth) and the juice expressed with a wooden masher, then cooled by immersion of the receiving flask in running water. The pink, slightly turbid filtrate, now about 300 cc per pound of meat, is introduced into cellophane casings‡ each about one meter in length and suspended in the upper two-thirds of a suitable tall, narrow vessel against 2 vol-

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1 Stock, A. H., *J. Immunol.*, 1939, **36**, 489; *J. Biol. Chem.*, 1942, **142**, 777.

2 Todd, E. W., and Hewitt, L. F., *J. Path. and Bact.*, 1932, **35**, 973.

† No difference in the rate or amount of growth was observed when New York City tap water was compared with distilled water.

‡ Visking Cellulose Sausage Casing, "NoJax" 27/32.

TABLE I.
Comparison of Commercial Peptones.

Brand	% dialyzable	pH of dialysate	Order of increasing color of dialysate	Precipitate with phosphate	Order of decreasing value for growth	Cost per lb
Pfanstiehl	79	5.32	9 (B)	±	1	$4.10
Difco proteose	63	6.80	3 (Y)	±	2	6.00
" neopeptone	80	6.90	7 (B)	++	3	6.00
" bactopeptone	89	7.28	4 (Y)	0	4	4.85
" tryptose	78	7.15	5 (Y)	0	5	6.00
" tryptone	85	7.18	6 (Y)	0	6	4.50
Wilson bacteriological	89	5.57	2 (Y)	0	7	1.10
Witte	55	6.70	1 (Y)	++	8	*
Fairchild	85	5.43	8 (B)	0	9	*

B—Brown. Y—Yellow. *Not available.

umes of water outside. After 12-18 hours dialysis at 4°C, the outside water is replaced; this in turn is replaced after a similar interval for a third dialysis. In this way an estimated 90% of the dialyzable components are extracted into a volume six times the volume of the original filtrate.

If the pooled dialysate is not to be used immediately for media, it should next be concentrated *in vacuo* (15-20 mm Hg) to about 1/20 the volume. With care it may be possible to avoid the use of an antifoaming agent in this process; if one is required, it appears preferable to make small additions of ethyl alcohol rather than the more toxic and persistent octyl alcohol customarily employed.

On standing overnight in the ice box, this concentrate yields an abundant crop of creatine and creatinine crystals which may be removed by centrifugation and discarded. No difference could be detected in the quantity of growth supported by the medium when these crystals were eliminated from one portion and retained in another.

The concentrate may be stored for a period of at least eight months, or longer, by freezing in plastic containers in a dry ice box or by freezing and drying in high vacuum. The latter procedure appears to be the one of choice, despite some difficulty in the drying due to the high salt content with resultant hygroscopic nature, since portions of the dried material may readily be weighed to meet the varied requirements of different experiments.

A yield of about 8 g of the dried extract per 454 g (1 lb) of the original meat is obtained. Excellent growth is supported by 1 g of this material per liter of medium, or with frozen preparations, a quantity representing 1/8 lb of meat per liter. These quantities are at least twice the minimum requirements for optimal growth.

Purification of the Peptone. In Table I are listed various properties of different commercial peptones. It will be seen that there are wide differences in their capacities to support growth, in cost and in color, so that in any particular problem the choice must be governed by the relative weight assigned to these factors. It has, moreover, been observed (Fig. 1) that the different peptones cannot be consistently graded on the basis of growth rate and yield since their relative values in this regard are in part dependent on the particular strain of group A streptococcus employed.

An experiment shown in Fig. 1 measured turbidimetrically the growth of 5 strains of group A hemolytic streptococcus in dialysate media made with 9 different commercial peptones. At zero time each tube, containing 9 cc of medium, was inoculated with one loop of a 12-hour broth culture of organisms. Data are shown for the dialysate media (1 to 9) and for the stock Todd-Hewitt medium[2] made with unpurified Pfanstiehl peptone.

Of the peptones studied the 2 most useful for present purposes appear to be Pfanstiehl peptone and Difco Proteose. Both support excellent growth for all strains tested. The lower cost of the former recommends it for large scale uses in which economy is a more important consideration than freedom from colored impurities. These colored ma-

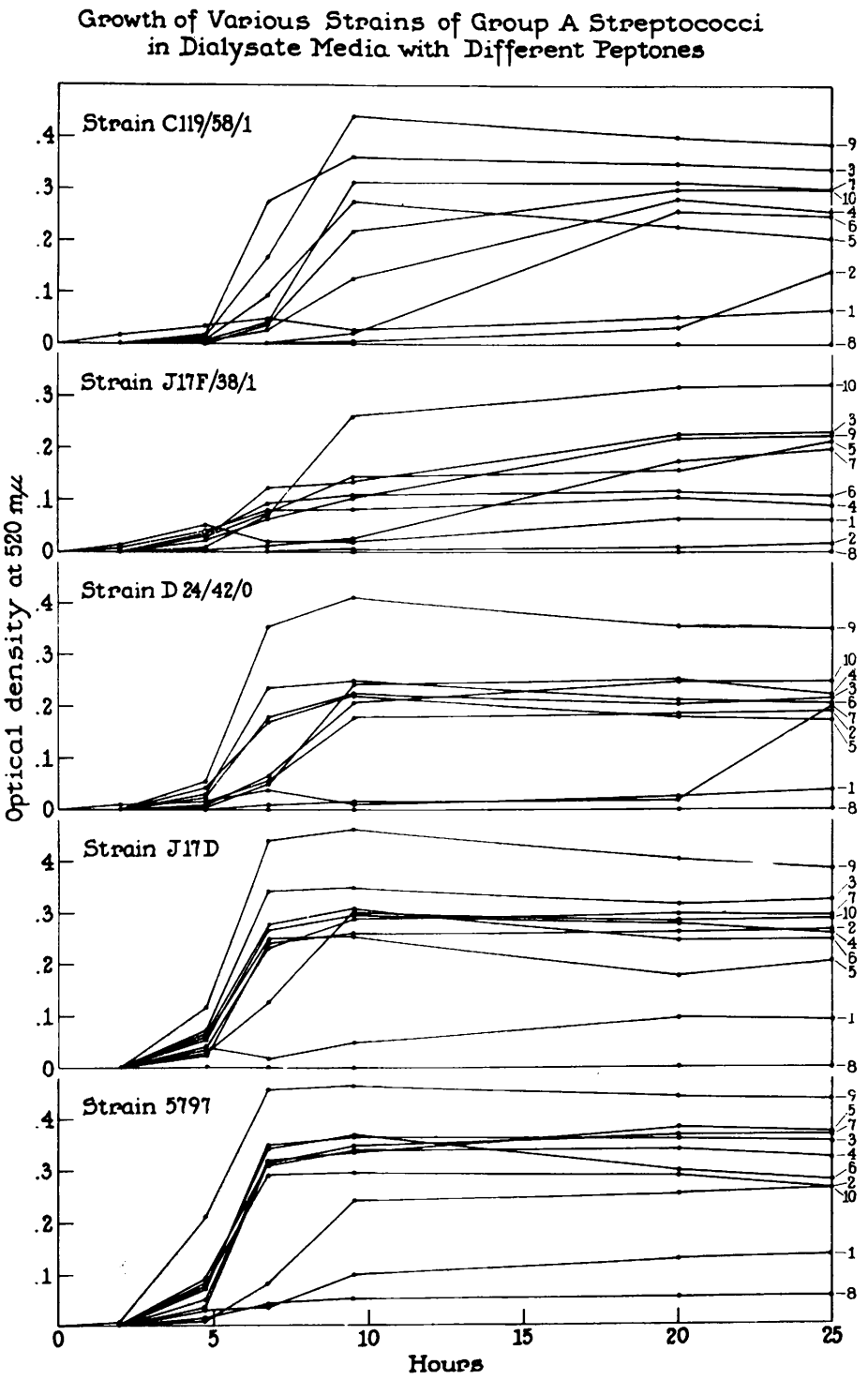

Fig. 1.

Growth of 5 different strains of group A hemolytic streptococci in dialysate media made with the following peptones: 1, Witte; 2, Wilson bacteriological; 3, Difco proteose; 4, Difco bactopeptone; 5, Difco tryptose; 6, Difco tryptone; 7, Difco neopeptone; 8, Fairchild; 9, Pfanstiehl; 10, Stock Todd-Hewitt medium with unpurified Pfanstiehl peptone.

terials can be reduced, but not eliminated, by repeated adsorption with charcoal at different pH's; what remain tend to persist through any subsequent fractionations of the culture fluid and may for this reason be found most objectionable. Proteose peptone, on the other hand, is initially of low color content and can be rendered almost colorless by charcoal adsorption of a 20% solution at pH 8.0. This step also removes the slight precipitate that sometimes develops at this alkalinity.

As a consequence of the high calcium content of neopeptone (Difco), the dialysate medium with this peptone becomes turbid after addition of the phosphate. If removed by preliminary filtration, the turbidity reappears during growth.

For purification the peptone is dissolved in water at 80°C to make a 20% solution, to which activated charcoal about 50 g per liter is added; the mixture is held at 80°C with stirring for one hour, then filtered hot through soft filter paper with the aid of suction. The clear filtrate is then dialyzed in a manner similar to that of beef heart extract.

After completion of dialysis, the pooled dialysate is adjusted to pH 8.0 and again adsorbed with charcoal 50 g per liter, overnight at 4°C. Following removal of the charcoal by filtration, the solution may be concentrated for storage by evaporation *in vacuo*, followed by freezing and drying. It is desirable to prepare as large a quantity of the dried purified peptone as possible in order to assure uniformity of experimental results. There appear to be uncontrolled variations in the qualities of different lots of the same brand of commercial peptone.

Preparation of the Complete Medium (Solid Content 2.3%). The following ingredients per liter of ultimate volume are dissolved in about 900 cc of water, adjusted to pH 8.0 with approximately N/1 NaOH, and then brought to a volume of 950 cc.:

Peptone: 15 g (or the dialysate from 20 g of original peptone)

NaCl: 2 g

Na_2HPO_4 (anhydrous): 0.5 g

This solution may be heat sterilized without impairment of the growth properties, although some undesired color and precipitate may be formed.

Just before use, the medium is completed by addition of 50 cc per liter of final volume of the following mixture, sterilized by filtration:

Beef heart extract: 1 g (or the dialysate from 1/8 pound of meat)

Glucose: 2 g

(Volume adjusted to 50 cc, pH to 8.0 at this point)

$NaHCO_3$: 2 g

Since the whole medium filters at about the same rate as water, it might be preferred to sterilize entirely by filtration. In this case, all ingredients are dissolved in their final concentration (the bicarbonate again being withheld until the pH is adjusted to 8.0) and the lot filtered.

In either case the medium should be inoculated immediately after completion; otherwise the pH may rise to 8.5 or higher in a few hours from decomposition of the bicarbonate. If delay in inoculation is unavoidable, the bicarbonate should be omitted and either added later or the pH during growth controlled by intermittent additions of 18 N NaOH.

It has been found possible, although not for our purposes advantageous, to combine the dried materials in the proper proportions so that complete medium may be formed merely by the addition of water. Such a procedure may be useful when it is necessary to use small amounts of a uniform medium at irregular intervals.

Discussion. Strains C119/58/1 and J17F/38/1, shown in Fig. 1, were selected for study because they had been found by Pappenheimer[3] to be the 2 most difficult to cultivate in the defined medium of Bernheimer, Gillman, Hottle and Pappenheimer.[4] Strains D24/42/0 and J17D were included because they had been found by Wilson[5] to require xanthine. Strain 5797 was currently in use for other purposes and was known to

[3] Pappenheimer, A. M., personal communication.

[4] Bernheimer, A. W., Gillman, W., Hottle, G. A., and Pappenheimer, A. M., *J. Bact.*, 1942, **43**, 495.

[5] Wilson, A. T., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 249.

grow abundantly on stock media. With all 5 of these strains Hartman[6] obtained light growth in the partially defined medium of Adams and Roe[7] which, following Wilson,[5] had been supplemented with horse serum and xanthine.

The dialysate medium evidently contains growth factors not present in sufficient concentrations in either of these semisynthetic media. No attempt has been made to fractionate the natural products further to elucidate the difference.

A potential advantage for the dialysate medium is the reduction in antigenic materials otherwise introduced with unpurified peptone and beef heart. It was found by Todd[8] that horse serum containing strong precipitating antibodies for some antigens in Proteose peptone failed to show any reaction with the corresponding dialysate medium.

Summary. A procedure is described for preparation of a medium containing only 2.3% solid material, all dialyzable. The medium has been found uniformly capable of supporting rapid and heavy growth of group A hemolytic streptococci.

The author is indebted to Dr. Rebecca C. Lancefield and to Miss Dorothy Sloan for their criticism and help.

6 Hartman, T., personal communication.

7 Adams, M. H., and Roe, A. S., *J. Bact.*, 1945, **49**, 401.

8 Todd, E. W., personal communication.

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Serum Levels and Excretion Studies in Mice Following Injection of a Penicillin-Albumin Complex.

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In a previous paper[1] mention was made of the fact that penicillin administered intramuscularly to mice in the form of a penicillin-albumin complex was eliminated more slowly than solutions of penicillin in water. Since the publications by Romansky and Rittman[2,3] have shown that penicillin suspended in peanut oil is excreted more slowly than solutions in saline and that when beeswax is added, in concentrations by volume ranging from 0.75 to 6%, the rate of excretion is further reduced, it seemed of interest to compare the efficacy of a penicillin-albumin complex with suspensions of penicillin in oil and in oil-beeswax.

Method. In the studies to be described comparison of serum levels and rates of excretion was made following intramuscular injection of an aqueous solution of a penicillin-albumin complex, suspensions of a calcium salt of penicillin in peanut oil and in peanut oil containing 2, 3 and 5% beeswax* and solutions of penicillin in distilled water.

Since earlier studies on rabbits had shown that serum levels and excretion rates on individual animals frequently varied widely, pooled serum and urine from groups instead of from individual animals were used. The method consisted of the intramuscular injection of penicillin in volumes from 0.2 to 0.5 ml into groups of 30 to 80 mice. The dosage in different experiments ranged from 20 to 400 Oxford units per 20 g mouse. For the excretion studies the first urine col-

1 Chow, B. F., and McKee, C. M., *Science*, 1945, **101**, 67.

2 Romansky, M. J., and Rittman, G. E., *Science*, 1944, **100**, 196.

3 Romansky, M. J., and Rittman, G. E., *Bull. U. S. Army Med. Dept.*, 1944, No. 81, 43.

* These suspensions were kindly supplied by Dr. A. E. Jurist of Products Development Laboratories of E. R. Squibb & Sons. The suspensions were made on a weight volume basis from commercial penicillin.