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.

lection was usually made one-half hour after injection and the second one-half hour later. Thereafter, collections were made at hourly intervals over periods of time ranging from 7 to 14 hours depending upon the nature of the preparation and the dosage given. Each hourly sample consisted of the pooled urine from the total number of mice in the group. The mice were picked up one at a time and the urine collected in a test tube. Urination was usually spontaneous but gentle pressure on the abdomen aided excretion. No special effort was made to empty the bladders of the mice before beginning the experiment. However, handling of the mouse during injection usually caused urination. Early samples were diluted 1-10 with distilled water and passed through Swinney (Seitz type) filters. Later samples were filtered undiluted.

For determination of penicillin concentrations the urine samples were further suitably diluted and tested, first by a 2-fold serial dilution test to determine the approximate concentration, and later by a more precise test in which small amounts (0.1 to 0.03 ml) of suitable dilutions were added to tubes containing 2 ml of a 10^{-6} dilution of a *Staphylococcus aureus* (Heatley) culture. Readings were made on the basis of turbidity after incubation for 18 hours at 37°C, the end point being the highest dilution showing complete inhibition. If, however, there were dilutions showing partial inhibition, the end point was considered to be midway between those showing complete and those showing partial inhibition. The potency was determined by comparison with a standard solution of crystalline penicillin G. By the 2-fold serial dilution test it was possible to determine as little as 0.04 units per ml, but by the more precise test it was not possible to show amounts less than 0.15 to 0.2 units per ml. The highest concentration tested by this method was a 1-20 dilution, while by the serial dilution test it was 1-4.

In the serum level studies the same time intervals for collection were used as in the excretion studies. Five or 6 mice were bled to death from the heart at each time interval and the samples of blood pooled. One-half

TABLE I.

Comparative Rate of Excretion of Penicillin in Urine of Mice Following Intramuscular Injection of a Penicillin-albumin Complex, a Suspension of Penicillin in Peanut Oil, and Solutions of Penicillin in Water.

Hr. after injection	Penicillin-Albumin complex				Cal. salt of penicillin in oil				Crystalline penicillin G aqueous sol. Cal. salt of penicillin aqueous sol.							
	Vol. urine, ml	O.U./ml urine	Total O.U.	%	Vol. urine, ml	O.U./ml urine	Total O.U.	%	Vol. urine, ml	O.U./ml urine	Total O.U.	%	Vol. urine, ml	O.U./ml urine	Total O.U.	%
½	3.3	44	145	19.3	0.85	154	131	17.5	0.35	1076	377	50.3	0.8	519	415	55.3
1	1.2	75	90		0.5	144	72		0.4	218	82.7		0.4	97	38.8	
2	1.6	55	88		1.2	46	55		1.8	22	39.6		2.4	5.6	13.4	
3	.9	39	35		1.2	12.8	15.4		1.3	1.58	2		1.7	0.88	1.5	
4	1.0	21	21		1.3	4.0	5.2		1.4	0.21	0.3		1.7	0.15	0.26	
5	1.0	11.3	11.3		1.0	1.2	1.2		1.7	0.1	0.17		1.4	0.08	0.11	
6	1.2	4.2	5		1.35	0.48	0.65		2.0	0.08	0.16		1.3	0.04	0.05	
7	.9	2.0	1.8		1.4	0.24	0.34		1.2	<0.03			2.0	<0.03	0.06	
			397.1	53			280.79	37.4			506.43	67.5			469.18	62.5

Thirty mice in each group received doses of 25 Oxford units per mouse.

to three-quarters ml could be obtained from each mouse. Blood was collected over periods of 4 to 10 hours depending upon the expected concentration of penicillin. The blood was allowed to clot, was then centrifuged, and the clear serum collected for test. Penicillin assay on the serum samples was done in the same manner as on the urine samples.

The penicillin-albumin complex was made by equilibrating a solution of crystalline penicillin G with human albumin as described by Chow and McKee.[1]

Results. In the first experiments, 4 groups of 30 mice received intramuscular injections of 25 Oxford units per mouse of a penicillin-albumin complex, of a calcium salt of penicillin in peanut oil, of solutions in water of crystalline penicillin G or of a calcium salt of penicillin. Urine was collected over a period of 7 hours and penicillin determinations made. No investigation of serum levels was made. The results are shown in Table I.

From the 2nd to the 7th hour higher concentrations of penicillin were present in the urine of those mice which received the penicillin-albumin complex and the penicillin in oil than in those which received solutions in water. The reverse was true one-half hour after injection. In those mice which had received penicillin in water approximately one-half of the total amount of penicillin injected was excreted during the first half hour.

In experiments with mice it is not possible to collect the total amount of urine excreted but by making frequent collections there is probably little loss. In Table I the volumes collected at the different time intervals and the total excretion in Oxford units is given. When aqueous solutions of penicillin were used and excretion was rapid the total recoveries in the 2 experiments were 67.5 and 62.5%. When a penicillin-albumin complex was used the total recovery was 53%, and when a calcium salt in oil was used recovery was 37.4%. In another experiment, not shown in Table I, in which 400 Oxford units in a 5% beeswax-oil mixture were injected, and excretion was greatly retarded, the total recovery was only 13.7%. It would seem that total recovery of penicillin was reduced as elimination from the body was retarded.

In further experiments a penicillin-albumin complex in 4 different dose levels, 25, 100, 200 and 400 Oxford units per mouse, was compared with like doses of aqueous solutions of penicillin. In all cases the penicillin-albumin complex retarded elimination. In general, like concentrations of penicillin were present in the urine 3 to 4 hours later in those mice injected with the penicillin-albumin complex than in those receiving aqueous solutions of penicillin.

A series of experiments was carried out in which a penicillin-albumin complex was compared with a calcium salt of penicillin in water, in peanut oil, and in peanut oil containing 2, 3 and 5% beeswax. Comparative serum levels and excretion rates were obtained following injection of 400 Oxford units per mouse. All results are expressed in units per ml of serum or urine.

In 2 out of 3 experiments with aqueous solutions, no penicillin was demonstrated in the serum 2 hours after injection; in the third experiment 0.06 unit was still present at this time but none one hour later. With the calcium salt suspension in peanut oil, penicillin (0.33 and 0.17 unit) was still demonstrable in the serum in 2 out of 3 groups at 4 hours; later tests were not made. With the 2% beeswax-oil, the last test was made 5 hours after injection, at which time the serum showed 0.39 unit. With the 3% beeswax-oil, the last test made at 10 hours showed 0.1 unit, and with the 5% beeswax-oil there was 0.17 unit at 9 hours and a trace at 13 hours. With the penicillin-albumin complex, 0.036 unit was present at 5 hours but none at 6 hours. Since it is generally agreed that less than 0.05 unit per ml is an effective inhibiting serum level, neither the oil nor the oil-beeswax experiments were carried out for a sufficient length of time. It would seem, however, that the penicillin-albumin complex was less effective even than 2% beeswax in oil in prolonging effective serum levels.

The figures on excretion indicate that the retarding effect of the penicillin-albumin complex was about the same as that of penicillin in oil. When beeswax was added to

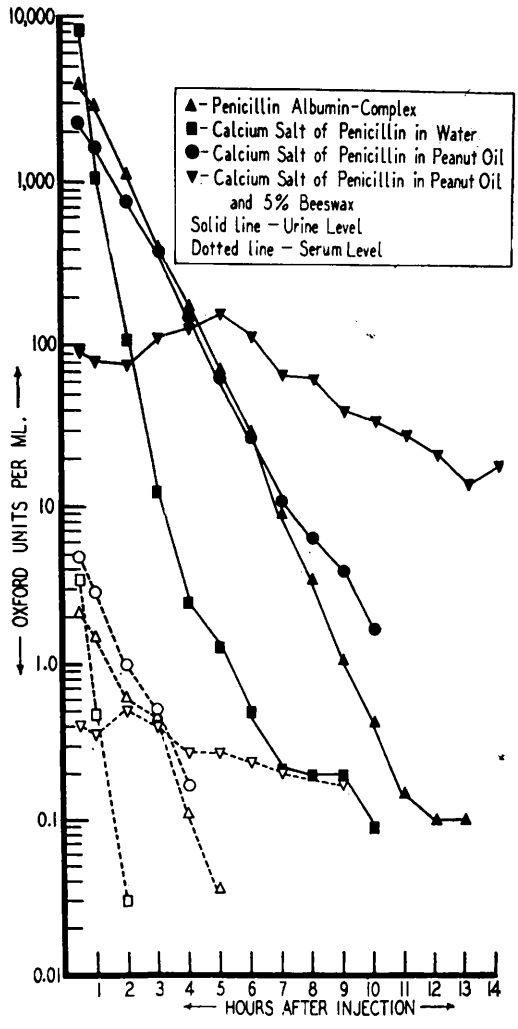

Fig. 1.

the oil the excretion rate was still further retarded, the degree of retardation being increased as the per cent of beeswax was raised. With the penicillin-albumin complex, the suspensions in oil, and solutions in water, the peak of excretion was reached one-half hour after injection. When 2% beeswax was added to the oil the peak in one experiment was still reached at one-half hour but in another experiment the peak was one hour after injection. With 3 and 5% beeswax the peaks of excretion came 3 to 5 hours respectively after injection. As with the serum level studies, experiments were not continued for a sufficient length of time to observe final elimination of penicillin.

Tests made 10 hours after injection of 400 Oxford units of the various preparations gave the following results in units per ml of urine: aqueous penicillin (3 experiments) 0.18, 0.08 and <0.02, suspension in oil (3 experiments) 3.6, 1.5, and 0.15, 2% beeswax-oil (2 experiments) 11.6 and 9.3, 3% beeswax-oil 19.2, 5% beeswax-oil 36.7, and a penicillin-albumin complex 0.44. Tests made 14 hours after injection of the 2, 3 and 5% beeswax-oil suspensions showed 5.7, 10.2 and 19.8 units per ml respectively and 23 hours after injection of 3 and 5% beeswax-oil suspensions 2.8 and 6.6 units respectively were demonstrated. Thus with both 3 and 5% beeswax in oil, penicillin was demonstrable in the urine 23 hours after injection in larger amounts than at 9 to 10 hours after injection of the penicillin-albumin complex. Fig. 1 shows the similarity of blood levels and excretion rates following injection of a penicillin-albumin complex and a suspension of a calcium salt of penicillin in oil. The more rapid elimination when an aqueous solution was injected and the much greater retention when a suspension in oil containing 5% beeswax was used are also shown. Where several experiments were done with the same preparation the figures were averaged.

The results in mice using suspensions of penicillin in oil and in oil-beeswax are in essential agreement with those of Romansky and Rittman who used rabbits as the experimental animals and extended their investigations to man. Richardson, Miller and Ahlgren,[4] using dogs, found slight retardation of absorption when a penicillin suspension in oil was given subcutaneously. Nelson[5] injected 2 human subjects intravenously with 35,000 and 63,000 units of a penicillin-albumin complex and found no prolongation of the action of penicillin. Intramuscular injection in man has, we believe, not been tried.

Summary. A method is described for the use of groups of mice in serum level and excretion studies on penicillin.

A slight prolongation of penicillin in the body of the mouse resulted from the intra-

[4] Richardson, A. P., Miller, I., and Ahlgren, M. W., unpublished.

[5] Nelson, R. A., personal communication.

muscular administration of a penicillin-albumin complex and a suspension of penicillin in peanut oil. When beeswax in 2, 3 and 5% concentration was added to the penicillin-peanut oil mixture the presence of penicillin in the body was greatly prolonged, the degree of prolongation being greater as the concentration of beeswax was increased.

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Heterophile Antibody Following Administration of Blood Group-Specific Substances.

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(Introduced by David Rapport.)

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In the course of studies on the production of isoagglutinins in human volunteers an agglutinin for sheep cells was encountered in the sera of a number of persons receiving group-specific A substance (Witebsky) and group-specific AB substance (Witebsky).* Twenty-three student volunteers of Tufts College Medical School participated. Sixteen belonging to blood groups B and O received 0.17 to 1.0 mg of A substance intravenously. Blood samples were taken before injection and again 2 weeks after. Sheep cell agglutinins were studied by the method of Paul and Bunnell.[1] An increase in titre of the second serum of 3 tubes or more above the titre of the first was observed in 7 volunteers, 9 remained unchanged or showed less definite increases. Seven volunteers belonging to blood group A received 0.05 mg of AB substance intravenously. Of these only one showed an increase of just 3 tubes.

Since A and AB substances are used to neutralize the isoagglutinins of O blood used for transfusion, the question arose of whether individuals transfused with such blood also developed sheep cell agglutinins. Blood sera were obtained before and one week after transfusion of 15 individuals and compared by the Stuart method.[2] Unlike the student volunteers, the members of this group received A and AB substances combined. Eight belonged to blood groups B and O; of these 5 showed an increase in titre of 3 tubes or more. Seven belonged to blood group A and of these one showed a definite increase.

Volunteers and transfusion recipients whose sheep-cell agglutinin titres were low before injection showed more spectacular rises than those with initially elevated titres. Of 27 individuals, whose original titres were 1/40 or less, 14 showed an increase of 3 tubes or more. Of 11 with original titres above 1/40, only one showed an increase.

The antibody found in these sera differs from the antisheep antibody found in the sera of individuals with infectious mononucleosis in that it can be absorbed with A substance, while the latter cannot. Further attempts to identify this heterophile antibody by absorption with guinea pig kidney and with boiled beef cells have so far yielded equivocal results.

We are indebted to Dr. Ernst Witebsky for valuable advice, and to Miss Lillian Rodofsky for extensive technical assistance.

* The group-specific AB substance was supplied by Eli Lilly & Company; the purified A substance was supplied by Sharp & Dohme.

1 Bray, W. E., *Synopsis of Clinical Laboratory Methods*, pp. 279-281.

2 *Diagnostic Procedures and Reagents*, 2nd Edition, 1945, pp. 449-456.