dosages, even up to 10,000 units per cc, were of no value and all animals died.

Discussion. The evidence presented here indicates that penicillin, with no protective coating or buffering materials, may be successfully administered to mice by mouth and, provided that sufficiently large quantities are given, protection is afforded against *E. rhusiopathiæ*. Since 50 cc of water containing 1000 units/cc was consumed by 5 mice in 48 hours, the effective average intake per mouse was 5000 units/day. This is approximately 5 times the amount used by Heilman and Herrell[1] in injecting the material. Penicillin is effective in the treatment of *Erysipelothrix rhusiopathiæ* infection if given early enough after exposure. Our results on this point are in accord with the findings of Heilman and Herrell[1] who showed protective action by the injection method of administration when begun 21 to 24 hours after challenge. Under our experimental conditions the effective time interval was 24 hours or less, but in field conditions institution of therapy at such an early stage of infection may be unattainable. It would seem, therefore, that penicillin may be of considerable value only in the control of the outbreak of this infection in domestic animals. The ease of oral administration makes its use feasible on a far greater scale than would be possible where injections are necessary.

Summary. Penicillin is shown to be of value in the protection of mice against experimental *Erysipelothrix rhusiopathiæ* infection.

Oral administration is successful in the treatment of mice even without any protective action from oil coating, or buffers, provided it is instituted within 24 hours after exposure. The use of penicillin in drinking water is found to be as effective as is its addition to the feed. Oral administration compares favorably with parenteral injection although larger amounts are required.

15171

Preparation of Human Typing Sera by Iso-immunization of Human Donors with Group Specific Substances.

LOUIS A. KAZAL, CLARA MATT, L. J. WENGER, AND RICHARD H. BARNES.

From the Department of Biochemistry, Medical Research Division, Sharp and Dohme, Glenolden, Pa.

Typing sera with extremely high agglutination titers have been prepared by Witebsky and his coworkers[1] in human donors following the intravenous injection of solutions containing minute amounts of group A and B specific substances. Wiener,[2] employing intramuscular injections of autoclaved saliva from group A and B individuals (secretors) instead of isolated group specific substances, confirmed Witebsky's observations. Pillemer *et al.*[3] have prepared as by-products of plasma fraction, concentrated isohemagglutinin globulins that were characterized not only by their high titer, but by their high avidity as well, *i.e.*, by the speed with which the serum agglutinated red cells. To the best of our knowledge, an investigation of the change in the avidity of typing sera following isoimmunization of human donors with group specific substances has not appeared in the literature. In the course of studies on blood group specific substances, we have had the opportunity to make such observations.

Nine subjects, 3 belonging to each of the following groups, O, A, and B, received intravenously 0.5 cc of a solution of group A and B

1 Witebsky, E., Klendshoj, N. C., and McNeil, C., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 167.

2 Wiener, A. S., Soble, R., and Polivka, H., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 310.

3 Pillemer, L., Oncley, J. L., Melin, M., Elliot, J., and Hutchison, M. C., *J. Clin. Invest.*, 1944, **23**, 550.

TABLE I.
Avidities and Titers of Blood Grouping Sera Prepared by Immunization of Donors with Group Specific Substances.

Serum preparation		Avidity (seconds)				Agglutination titer			
		Group "B" cells		Group "A" cells†		Group "B" cells		Group "A" cells†	
		*	**	*	**	*	**	*	**
Group "A"	No. 47	20/65	3/10			1:8	1:256		
	79	35/120	15/45			1:2	1:32		
	114	27/75	5/17			1:16	1:128		
	Pooled	20/50	4/8			1:16	1:256		
Group "B"	No. 73			15/30	10/25			1:32	1:64
	19			20/40	10/20			1:32	1:64
	98			12/20	5/12			1:32	1:128
	Pooled			12/40	5/9			1:16	1:128
Group "O"	No. 5	27/75	5/15	—	5/15	1:16	1:512	1:32	1:256
	41	—	3/10	10/30	5/15	1:32	1:1024	1:16	1:1024
	46	15/60	5/20	5/15	2/3	1:16	1:256	1:16	1:128
	Pooled	15/40	3/5	10/30	3/5	1:16	1:1024	1:64	1:1024

* Before immunization.
** After immunization.
† Sub-group not determined.

specific substances (0.2 mg A + 0.2 mg B). Three weeks after the injection the individuals were bled (500 cc) and their sera (stored under aseptic conditions in the refrigerator) were examined for titer and avidity. These were compared at the same time with control samples of sera (10 cc bleedings) obtained 1 week prior to the injection of group specific substances. The titers were determined by the serum dilution technic with 1% suspensions of washed (once) red cells. The agglutination response of the serum dilutions was observed after a 15-minute period of incubation at room temperature, followed by centrifugation for 1 minute at 2000 r.p.m. Readings were made macroscopically, and the endpoint reported as the greatest dilution giving a 4+ reaction, *i.e.*, complete agglutination. Avidity determinations* were obtained by mixing equal volumes of a 10% suspension of once-washed red cells and serum on a slide, which was placed on a slide-agglutination viewing box.[4] The viewing box was rocked slowly while observations were made macroscopically for the time required for the earliest visible agglutination and then for complete agglutination to develop.

The results that are summarized in Table I show the avidities and titers of the individual and the pooled lots of sera. All of the individual bleedings (numbered sera) were assayed at one time against the same red cell suspensions; the pooled sera of each group were assayed approximately one week later with other suspensions of red cells. There was a definite increase in the avidity and titer of the sera obtained from the immunized donors. The greatest increases in titer were observed in those individuals belonging to groups A and O. In the latter group sera were obtained that gave a 4+ reaction at a dilution of 1:1024 for both the anti-A and anti-B agglutinins. If the agglutination end point was taken as the greatest dilution showing some signs of agglutination, the titers of the more active sera would have values in the neighborhood of 1:4096. On the basis of a 4+ agglutination endpoint the titers obtained in our sera are at least as high as those obtained by Witebsky *et al.*[1] However, due to the variability of the quantitative agglutination test among different laboratories, such a comparison cannot be made with any certainty.[5] The greatest avidity response was obtained with

* We are indebted to Dr. L. K. Diamond of Harvard University for the technic of this determination and for the slide-agglutination viewing box that was employed.

4 Diamond, L. K., *J. Lab. Clin. Med.*, 1945, **30**, 204.

5 DeGowin, E. L., *J. Clin. Invest.*, 1944, **23**, 554.

the anti-B agglutinins of group A and O individuals. The pooled sera† in almost every case were found to have titers and avidities approximating those of the strongest sera in the group. However, this point cannot be stressed much since the pooled sera were assayed against a different batch of red cells.

Conclusions. The increase in agglutination titer following immunization confirms the work of Witebsky[1] and Wiener.[2] In addition it has been shown that immunization with group specific substances increases the avidity, and that this increase roughly parallels the increase in agglutinin titer.

† We are very grateful to Dr. Wm. C. Boyd of Harvard University for comparing our pooled sera with the Harvard reference standards[5] obtained as byproducts of plasma fractionation. The titers and avidities obtained by Dr. Boyd compare favorably with the reference standards. We also wish to thank Dr. Ernest Witebsky for kindly confirming the results of our assays.

15172

Relationship of Turbidity to Acid Production by *Lactobacillus arabinosus.*

HARRIS ISBELL.* (Introduced by F. S. Daft.)

From the Division of Physiology, National Institute of Health, Bethesda, Md.

In microbiological assays using the *Lactobacillus arabinosus* either turbidity or total acid production may be used as a measure of the response to the organism to various growth factors. Since, in all tests for which the organism is used, the turbidity resulting from bacterial growth increases with the amount of acid produced, it has generally been assumed that the turbidity corresponding to a given amount of acid production would be the same regardless of the growth factor to which the organisms were responding. In the course of some determinations of nicotinic acid, pantothenic acid, and biotin it was noted that the turbidity was much greater in relation to acid production when the organisms were responding to suboptimal amounts of nicotinic acid than when responding to suboptimal amount of biotin or calcium pantothenate.

Methods. All tests were carried out in triplicate except those involving *l*-tryptophane† which were done in duplicate. The organisms were grown in Evelyn photometer tubes in 10 cc of the medium of Snell and Wright[1] as modified by Krehl, Strong, and Elvehjem,[2] omitting nicotinic acid, biotin, pantothenate, or *l*-tryptophane as the case might be. Stock cultures were carried and organisms were prepared for use as inocula as described by Snell and Wright.[1] Turbidity was measured in the Evelyn photoelectric colorimeter using filter 660. The total acid produced was titrated with 0.1 N sodium hydroxide. Lactic acid was determined by the method of Barker and Summerson.[3] These determinations were carried out in the biotin, nicotinic acid, and pantothenate tests after 18, 42, and 66 hours incubation at 37°C. In the tryptophane tests only the 66-hour determination was made and lactic acid production was not measured. The response to nicotinic acid was studied over the range from 0.1 to 1.0 μg, biotin from 0.1 to 2.0 millimicrograms, calcium pantothenate from 0.02 to 0.5 μg, and *l*-tryptophane from 2 to 10 μg per 10 ml of medium.

* Present address: U. S. Public Health Service Hospital, Lexington, Ky.

† We are indebted to Dr. J. G. Wooley for these data.

1 Snell, E. E., and Wright, L. D., *J. Biol. Chem.*, 1941, **139**, 675.

2 Krehl, W. A., Strong, F. M., and Elvehjem, C. A., *Ind. Eng. Chem., Analyt. Ed.*, 1943, **15**, 475.

3 Barker, S. B., and Summerson, W. H., *J. Biol. Chem.*, 1941, **138**, 535.