of a Vi-phage and a non-specific antityphoid phage in a urinary typhoid carrier, only the Vi-phage appeared in the urine in amounts greater than those administered. The numbers of *B. typhosus* in the urine were transiently decreased as the result of phage-treatment but increased again considerably with the appearance of W-form and lysogenic V-forms in addition to the typical V-forms which had been persistently present. The presence of W- and lysogenic V-forms was attributed to the action of V-phage in the bladder-urine and the presence of the V-form organisms due to the intermittent discharge of organisms from a focus in which the action of the Vi-phage was probably inhibited. At the same time specific antibodies against both phages were developed in the blood. The type-specificity of the phage and bacteriophagic typing of the Vi-form organisms repeatedly isolated from the urine remained constant.

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Studies on Stability of Dilute Purified Tuberculins.

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Concentrated tuberculin is remarkably stable whereas diluted tuberculin usually undergoes rapid deterioration. By the use of alkaline buffer solutions as diluting agents, however, old tuberculin has been claimed to maintain its potency for 4 to 6 months.[1, 2, 3] On the other hand, when the same diluents were applied to purified tuberculins by the same authors less favorable results were obtained; deterioration of the product generally occurring in 4 to 6 weeks. It is evident that a suitable diluent has not been found. In the present report a phosphate buffer of pH 7.0 is described which in our hands has been found to maintain the potency of dilute purified tuberculins for at least 4 to 6 months.

Materials and method. The preparation of the purified tuberculin employed for the study was similar in principle to that of Seibert's PPD. Its potency and other properties will be reported elsewhere. For the present it is sufficient to state that comparative tests made on

1 Douglas, S. R., and Hartley, P., *Tubercle*, 1934, **16**, 105.

2 Gottschall, R., and Bunney, W. E., *J. Immunol.*, 1938, **34**, 103.

3 Jensen, K. A., Bindslev, G., Moller, S., Hansen, A., and Lind, P., *Tubercle*, 1938, **19**, 385.

several hundred human subjects between our product and the PPD kindly supplied to us by Dr. Florence Seibert revealed no difference in activity. Only the first dose of the Mantoux test consisting of 0.00002 mg in 0.1 cc was used as a measurement of biological activity. It was thought that the result obtained with this strength would be representative of higher concentrations. The purified tuberculin was diluted with buffered saline, the method of preparation of which was as follows: To each liter of normal saline is added 20 cc of a stock buffer solution consisting of 62.5 g of $Na_2HPO_4.12H_2O$ and 14.4 g of $NaH_2PO_4.2H_2O$ in 500 cc of distilled water. The buffered saline has a pH of 7.0 and is sterilized by autoclaving at 122°C for 20 minutes. After cooling, 40 cc of 5% phenol is finally added to effect a 0.5% concentration. Purified tuberculins were accurately measured, diluted in this buffered saline and distributed in 20 cc bottles fitted with rubber caps. One set was kept at room temperature (20°C) and the other in the refrigerator (8°C). Testings were done entirely on young subjects between the age of 9 to 13 years by intracutaneous injections made on the dorsal surface of the forearm. Readings were made 48 hours afterwards. When comparative tests were made, one injection was given in each arm in approximately the same position. A set of syringes and needles was kept entirely for this purpose. Final testings (data presented in Tables I and II) were done with new syringes and needles. For comparison we used freshly prepared solutions of Seibert's PPD. The dilute tuberculin kept at room temperature was tested weekly for the first month and every other week thereafter on a group of 13 volunteers. Only one injection was given at each testing. The tuberculin kept at the lower temperature was tested at longer intervals.

Dilute tuberculins kept at 20°C. The nature of the reaction from week to week of individuals repeatedly tested remained almost constant. The results of tests made at the end of 4 months of the same subjects and 17 new ones are presented in Table I. Parenthetically it may be stated that the stability of the dilute tuberculin kept at room temperature greatly exceeded our expectation. The experiment was concluded at this time owing to the exhaustion of our testing material. From the data it is clear that the agreement between the dilute purified tuberculin and the freshly prepared PPD of Seibert's as a whole is close and the slight differences recorded are well within experimental errors. It is true that one subject was missed by the old material. On the other hand, 2 were also missed by the new material. Unfortunately, these subjects could not be retested. From our experience this irregularity is not uncommon among young ado-

TABLE I.

Comparison of Sizes of Reaction Between Freshly Prepared PPD and Diluted Purified Tuberculin kept at 20°C for 4 Months.

Name Tuberculin	Repeatedly Tested Subjects. LCH	FWH	HPY	TK	FPC	CMJ	LPC	SCK	SCL	CJK	WH	CHJ	HCC
†Purified	25*	17	10	14	15	7	10	16	15	10	12	12	8
‡Seibert's	23	13	8	14	15	7	22	18	16	15	14	12	12

Name Tuberculin	New Subjects. LSC	KSC	SWY	MTS	PWT	CSC	KCL	KFH	CH	LCF	CCC	CCT	CMH	LCM	LHM	TYS	MCC
†Purified	10	10	7	5	3	8	13	10	0	3	8	8	9	13	0	0	0
‡Seibert's	5	6	0	3	0	15	15	12	10	10	10	10	9	13	0	0	0

TABLE II.

Comparison of Sizes of Reaction Between Freshly Prepared PPD and Diluted Purified Tuberculin Kept at 8°C for 6 Months.

Name Tuberculin	HCL	TCK	HS	TYC	SCL	WHC	WC	CHC	CHH	CYF	KF	WWJ	CYA	CPT	LPC	WHY	KKY	LHH	LCH	16 subjects
†Purified	25	20	20	15	15	7	22	22	20	13	12	10	12	11	10	5	7	7	0	0
‡Seibert's	22	16	12	10	13	5	30	25	25	20	20	20	15	15	12	12	10	10	10	0

*All figures are in millimeters and represent diameters of reactions.
†Kept at temperature and time indicated.
‡Freshly prepared PPD.

lescents. Usually, however, retesting would correct these differences. Incidental observation was also made on Seibert's PPD kept at room temperature for 3 months. It was found that the reactions were similar to those elicited by the fresh preparation and thus indicated no deterioration.

Dilute tuberculin kept at 8°C. This was tested at the end of 3 and 6 months. Since the first result showed complete agreement with a fresh preparation of Seibert's PPD in 8 parallel tests it is necessary only to present the data obtained at the latter date. The results given in Table II show the comparative sizes of the reactions between the purified tuberculin and the PPD among 35 tested individuals. It can be seen from the table that there is a general agreement but the freshly prepared PPD of Seibert's elicited a stronger reaction in a larger number of positive reactors. However, the stability of the dilute purified tuberculin kept for 6 months at 8°C seems to be well established.

Comment. From the results presented above, 3 points are of interest. (1) Repeated endermal injections of human subjects over a period of 4 months with 0.00002 mg of our purified tuberculin each time, as expected, did not alter the sensitivity of the skin. This is shown by the remarkable constant reactions observed in the same individuals so tested. In other words it indicates that our purified tuberculin in the doses employed is not sensitizing. (2) The general impression that dilute purified tuberculin undergoes rapid attenuation even in buffer solutions is not borne out by the present study. Indeed we have yet to observe either marked or complete destruction of its activity preserved in a neutral buffer when it was kept at 20°C for 4 months and 8°C for 6 months. Why the buffer solution employed by us has a better preservative effect for purified tuberculins than those employed by others is not clear. It is possible that a neutral pH is an important factor although we have, as yet, no data to support our contention. (3) The observation made by Jensen, *et al.*,[3] that phenol inactivates dilute tuberculin is not substantiated by our finding since the preservative employed in our buffer is phenol. Because the study is made on only 2 preparations (one of ours and one of Seibert's) it is premature to apply this conclusion to purified tuberculin in general. Still we believe the simplicity of the buffer and the preservative effect so far shown have much to recommend it for further trials in the field of purified tuberculin.

Summary. A phosphate buffer is described which has been found to maintain the skin-reactivity of dilute purified tuberculin for at least 4 months at 20°C and 6 months at 8°C.