alluded to but not expanded upon.[3] We have not hitherto concerned ourselves particularly with this specialized field and can only state that several of our patients made voluntary mention of the fact that their skin had become softer and less dry and that excoriations due to scratching had healed upon subsidence of the pruritus some whose pruritus had not entirely disappeared found that scratching failed to produce the excoriation which had been the result previous to treatment.

Though there is much yet to be done clinically and in the laboratory before the full implications of adenylic acid administration to human beings can be completely evaluated, we feel that the results to date are sufficiently good to make broader experimentation desirable, and hence to warrant passing on our experiences to other workers.

*Summary.* Thirty-six patients suffering from pruritus of diverse etiology were treated with adenylic acid. In thirty instances there was a subsidence of the pruritus ranging from complete to mild. So far we have been able to find in the rather extensive literature no reference to the beneficial effect of adenylic acid upon pruritus.

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## 17199. Chemotherapy of Leukemia. IV. Effect of Folic Acid Derivatives on Transplanted Mouse Leukemia.*

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Since the first report of promising results in the clinical treatment of acute leukemia with 4-amino-pteroylglutamic acid,[1] this drug[2,3] and two other related compounds[3,4] have been reported to be active against certain strains of transplanted mouse leukemia. These clinical and experimental findings suggested the advisability of screening a large number of compounds related to pteroylglutamic acid (PGA) against transplanted mouse leukemia. This series included not only compounds closely related in structure to folic acid, but also pyrimidines, pteridines and purines. The results of the preliminary testing of 90 such compounds are herewith reported.

*Method.* The technic for evaluation of the chemotherapeutic activity of a given drug by means of its ability to prolong the survival time of mice with transmitted leukemia has been described previously.[3]

In a typical experiment, 240 mice of the inbred Akm stock were injected intraperitoneally with 0.1 cc of a saline suspension of leukemic spleen so diluted that 0.1 cc contained 1,000,000 cells. Leukemia Ak 4,[5] a relatively acute strain, was used in these par-

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† Fellow of The American Cancer Society, recommended by the Committee on Growth of The National Research Council.

1 Farber, S., Diamond, L. K., Mercer, R. D., Sylvester, R. F., Jr., and Wolff, J. A., *New England J. Med.*, 1948, **238**, 787.

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5 Burchenal, J. H., Biedler, J. L., Nutting, J., Stobbe, G. D., to be published.

TABLE I.
Compounds Showing Definite Chemotherapeutic Activity Against Leukemia Ak 4.

| Compound | Dose mg/kg | Wt change (gr.) | | Survival time (days) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Untreated | | | | Treated | | | | |
| | | Untreated† | Treated* | No. Mice | Range | Mean | S.D. | No. Mice | Range | Mean | S.D. | % increase treated |
| 4-Amino-N$^{10}$-methyl-pteroylglutamic acid | 3 | +1.5 | +0.5 | 19 | 11-19 | 13.4 | ±1.78 | 10 | 28-39 | 30.1 | ±3.08 | 125 |
| | 3 | +2.3 | —0.7 | 20 | 10-16 | 12.4 | ±1.5 | 7 | 31-37 | 34.6 | ±1.84 | 179 |
| | 3 | +1.9 | +2.9 | 19 | 11-16 | 13.2 | ±1.57 | 8 | 26-45 | 31.9 | ±5.65 | 142 |
| | 3 | +1.9 | —1.2 | 20 | 11-18 | 13.5 | ±1.66 | 9 | 21-32 | 24.9 | ±3.57 | 84 |
| | 3 | +3.7 | +3.1 | 20 | 11-19 | 13.3 | ±1.78 | 8 | 30-43 | 34.2 | ±4.18 | 157 |
| | 3 | +4.9 | +2.4 | 20 | 10-15 | 12.7 | ±1.76 | 10 | 20-40 | 29.3 | ±5.44 | 131 |
| | 3 | +2.3 | +1.1 | 20 | 10-14 | 12.1 | ±1.26 | 17 | 20-36 | 27.0 | ±3.94 | 123 |
| 4-Amino-9-methyl pteroylglutamic acid | 3 | +1.7 | +3.5 | 20 | 10-14 | 11.7 | ±1.12 | 6 | 19-29 | 24.5 | ±3.74 | 109 |
| | 4 | +1.9 | +1.8 | 20 | 11-18 | 13.5 | ±1.66 | 9 | 22-28 | 24.2 | ±2.57 | 79 |
| | 4 | +2.9 | +2.6 | 19 | 11-17 | 12.6 | ±1.70 | 7 | 19-43 | 29.3 | ±6.95 | 133 |
| | 4 | +2.5 | +5.5 | 20 | 10-18 | 11.7 | ±1.85 | 7 | 21-32 | 26.0 | ±4.29 | 122 |
| | 4 | +3.3 | +1.1 | 19 | 11-16 | 12.9 | ±1.47 | 6 | 33-41 | 37.1 | ±3.18 | 188 |
| 4-Amino-9,10-dimethyl pteroylglutamic acid | 3 | +1.7 | +2.7 | 20 | 9-14 | 11.5 | ±1.24 | 10 | 22-34 | 29.5 | ±3.91 | 156 |
| | 5 | +1.9 | +1.0 | 20 | 11-18 | 13.5 | ±1.66 | 5 | 28-35 | 31.0 | ±2.68 | 129 |
| | 5 | +2.5 | +2.4 | 20 | 10-18 | 11.7 | ±1.85 | 5 | 32-38 | 34.2 | ±2.04 | 192 |
| | 5 | +3.3 | +0.9 | 19 | 11-16 | 12.9 | ±1.47 | 6 | 32-43 | 36.1 | ±3.58 | 180 |
| | 3 | +1.7 | —1.7 | 20 | 10-14 | 11.7 | ±1.12 | 6 | 28-42 | 34.2 | ±3.73 | 192 |
| | 3 | +0.3 | +0.5 | 16 | 10-14 | 12.2 | ±1.19 | 9 | 26-32 | 27.8 | ±1.81 | 128 |
| 2,6-Diaminopurine | 90 | +1.7 | +0.9 | 20 | 10-16 | 12.0 | ±1.32 | 8 | 17-31 | 23.7 | ±4.44 | 97 |
| | 90 | +0.8 | +1.0 | 20 | 10-15 | 11.7 | ±1.74 | 9 | 19-26 | 22.5 | ±2.50 | 92 |
| | 90 × 2<br>75 × 8 | —0.5 | +1.4 | 20 | 10-14 | 11.3 | ±1.28 | 8 | 21-31 | 26.1 | ±3.85 | 131 |
| | 100 | +2.3 | —1.4 | 19 | 11-16 | 13.3 | ±1.87 | 10 | 20-40 | 28.6 | ±6.22 | 115 |
| | 90 | +2.1 | —0.5 | 19 | 9-16 | 10.7 | ±1.83 | 18 | 12-24 | 18.4 | ±3.94 | 72 |
| | 100 | +1.0 | —0.6 | 20 | 10-33 | 14.5 | ±4.50 | 9 | 20-31 | 24.4 | ±3.38 | 68 |

* Weight change calculated as the difference between the initial weight and that two weeks later.
† Weight change calculated as the difference between the initial weight and that one week later.
S.D. = Standard deviation.

ticular experiments. Forty-eight hours later, these mice were divided into comparable groups of 10 mice each (2 sets of untreated controls, one set of controls treated with a standard compound of known activity, 4-amino-$N^{10}$-methyl-pteroylglutamic acid, and 21 sets of mice treated with unknown compounds). Compounds were given intraperitoneally in maximum tolerated doses 3 times weekly for 10 doses. Water soluble compounds were dissolved in saline. Substances insoluble in water were usually suspended in 5% gum arabic in saline. The results of treatment with an unknown substance were compared with those obtained with the standard compound, 4-amino-$N^{10}$-methyl-PGA which has previously been shown to possess a high degree of chemotherapeutic activity[3] against Ak 4 leukemia. Maximum tolerated dosage was used throughout in an attempt to procure the maximum effect. The mice were observed for the development of leukemia and autopsied at death. If gross evidence of leukemia was not conclusive, microscopic sections were taken. The rationale behind the various steps of this technic has been outlined in previous reports.[6]

*Results.* The derivatives of pteroylglutamic acid which show a definite chemotherapeutic effect against Ak 4 leukemia are listed in Table I. The compounds included here are only those which show approximately a doubling of the survival time of the treated animals as compared with the untreated controls. Experiments in which these compounds have been evaluated are listed in detail. Further data on two compounds previously reported[3,4] (4-amino-$N^{10}$-methyl-PGA and 2,6-diaminopurine) are shown in Fig. 1. Table II includes those compounds which have shown a suggestive effect by increasing the average survival time approximately 50%. Table III lists the compounds which have shown no evidence of chemotherapeutic activity after at least one satisfactory test.

*Discussion.* In man, some leukemias do and some do not respond to antifolic therapy and, similarly, in the mouse not all strains of

TABLE II. Compounds Showing Slight to Moderate Chemotherapeutic Activity Against Leukemia Ak 4.

| Compound | Dose mg/kg | Wt change (gr.) | | Survival time (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Untreated | | | | Treated | | | | |
| | | Untreated† | Treated | No. Mice | Range | Mean | S.D. | No. Mice | Range | Mean | S.D. | % increase treated |
| 4-Amino-pteroyl-aspartic acid | 30 | +3.7 | +4.6† | 20 | 11-19 | 13.3 | ±1.78 | 10 | 15-25 | 18.1 | ±3.18 | 36 |
| | 30 | +2.4 | +2.7* | 18 | 8-15 | 12.2 | ±1.61 | 8 | 17-28 | 21.5 | ±3.74 | 76 |
| | 30 | +1.9 | +2.4† | 20 | 11-18 | 13.5 | ±1.66 | 10 | 14-32 | 18.4 | ±5.07 | 36 |
| | 30 | +2.3 | —0.9* | 20 | 10-16 | 12.4 | ±1.50 | 9 | 15-26 | 20.2 | ±4.05 | 63 |
| | 30 | +3.3 | +0.6* | 19 | 11-16 | 12.9 | ±1.47 | 9 | 15-53 | 25.5 | ±11.23 | 98 |
| 4-Amino-3',5'-dibromo-pteroylglutamic acid‡ | 60 | +1.5 | +1.2* | 20 | 9-16 | 12.5 | ±1.87 | 9 | 14-33 | 19.3 | ±5.4 | 54 |
| | 70 | —0.9 | +1.9† | 19 | 10-22 | 13.5 | ±2.76 | 7 | 14-22 | 18.1 | ±2.54 | 34 |
| 4-Amino-pteroyl threonine | 200 | +0.3 | +1.3† | 15 | 10-14 | 12.2 | ±1.19 | 10 | 14-27 | 19.5 | ±4.46 | 60 |
| | 200 | +3.5 | +4.4* | 19 | 10-13 | 11.0 | ±1.24 | 9 | 15-22 | 19.0 | ±1.88 | 73 |
| 4-Amino-pteroyl-glutamic acid | 0.3 | +3.7 | +2.8* | 20 | 11-19 | 13.3 | ±1.78 | 8 | 15-25 | 20.0 | ±3.24 | 50 |
| | 0.3 | +3.3 | +2.5* | 19 | 11-16 | 12.9 | ±1.47 | 9 | 22-34 | 29.1 | ±3.24 | 126 |

[6] Burchenal, J. H., Lester, R. A., Riley, J. B., and Rhoads, C. P., *Cancer*, 1948, **1**, 399.

TABLE III.
Compounds Showing No Evidence of Chemotherapeutic Activity Against Leukemia Ak 4.

| Compound | Dose, mg/kg |
|---|---|
| *Pyrimidines* | |
| 2-Amino-4-methylpyrimidine | 750 |
| 2-Amino-4-(4'-arsonophenylamino)pyrimidine | 15 |
| 2-Amino-4-methyl-5-acetylpyrimidine | 64 |
| 2-Amino-4,5-dimethylpyrimidine | 250 |
| 2-Amino-4,6-diacetylaminopyrimidine | 175 |
| 2-Amino-4-hydroxy-5-(2',4'-dichlorophenoxy)pyrimidine | 1000 |
| 2-Amino-4-hydroxy-5,6-dimethylpyrimidine | 250 |
| 2-Amino-4-hydroxy-5-p-chlorophenoxy-6-methylpyrimidine | 1000 |
| 2-Amino-5-bromo-6-hydroxypyrimidine | 250 |
| 2,4-Diamino-5-methylpyrimidine | 75 |
| 2,4-Diamino-5-(2', 4'-dichlorophenoxy)pyrimidine | 250 |
| 2,4-Diamino-5,6-dimethylpyrimidine | 35 |
| 2,4-Diamino-6-methylpyrimidine | 125 |
| 2,4-Diamino-6-hydroxypyrimidine | 450 |
| 2,5-Diamino-4,6-dihydroxypyrimidine | 150 |
| 2,4-Dihydroxy-5-chloroacetamidopyrimidine | 50 |
| 2,4-Dihydroxy-5,6-diaminopyrimidine | 100 |
| 2,6-Dihydroxy-5-nitropyrimidine | 150 |
| 2,6-Dihydroxy-5-bromopyrimidine | 300 |
| 2,6-Dihydroxy-5-aminopyrimidine | 250 |
| 2,6-Dihydroxy-4,5-diaminopyrimidine | 15 |
| 2,4,6-Trihydroxypyrimidine | 35 |
| 2,4,5,6-Tetrahydroxypyrimidine | 250 × 2<br>125 × 7 |
| 2-Mercapto-4-hydroxypyrimidine | 100 |
| 2-Mercapto-4-hydroxy-5-methylpyrimidine | 1000 |
| 2-Mercapto-3-o-tolyl-4,6,6-trimethylpyrimidine | 350 |
| 2,4,6-Trichloropyrimidine | 15 |
| 2-Chloro-4-dimethylamino-6-methylpyrimidine | 0.75 |
| 2-Methyl-4-hydroxy-5-ethoxymethylpyrimidine | 750 |
| 1-Butyl-2-hendecyl-1,4,5,6-tetrahydropyrimidine | 4 |
| *Hexahydropyrimidines* | |
| 1,3-Bis(1,3-dimethylbutyl)-5-nitro-5-methylhexahydropyrimidine | 750 |
| 1,3-Bis(1,3-dimethylbutyl)-5-nitro-5-ethylhexahydropyrimidine | 300 |
| 1,3-Bis(2-ethylhexyl)-5-amino-5-methylhexahydropyrimidine | 35 |
| 1,3-Diisopropyl-5-amino-5-methylhexahydropyrimidine | 125 |
| 1,3-Dibenzyl-5-nitro-5-methylhexahydropyrimidine | 750 |
| 1,3-Dibenzyl-5-amino-5-methylhexahydropyrimidine | 32 |
| 1,3-Di-p-tolyl-5-amino-5-methylhexahydropyrimidine | 75 |
| *Pteridines* | |
| 2,4-Diaminopteridine | 100 |
| 2,4-Diamino-6-methylpteridine | 200 × 6<br>100 × 1 |
| 2,4-Diamino-7-methylpteridine | 60 |
| 2,4-Diamino-6-p-carboxyanilinomethylpteridine* | 125 |
| 2,4-Diamino-6-N-methyl-p-carboxyanilinomethylpteridine | 125 |
| 2,4-Diamino-6,7-dimethylpteridine | 50 |
| 2,4-Diamino-6,7-dihydroxypteridine | 500 |
| 2,4-Diamino-6,7-diphenylpteridine | 100 |
| 2,4-Diamino-6,7-dicarboxypteridine | 250 |
| 2,4-Diamino-6,7-bis(4-aminophenyl)pteridine | 500 |
| 2,4-Diamino-6,7-bis(p-sulfinomethylaminophenyl)pteridine | 400 |
| 2,4-Diamino-5,7-dihydroxypyrimido(4,5-e)pteridine | 300 |
| 2-Amino-4-hydroxypteridine | 1000 × 1<br>667 × 3 |
| 2-Amino-4-hydroxy-6-methylpteridine | 15 |
| 2-Amino-4,5,7-trihydroxypyrimido(4,5-e)pteridine | 300 |
| 2,4,6,7-Tetrahydroxypteridine | 10 |
| 2,4-Dihydroxy-6,7-dimethylpteridine | 250 |
| 2,4-Dihydroxy-6,7-diphenylpteridine | 125 |

| | |
|---|---|
| *Compounds directly related to pteroylglutamic acid* | |
| Pteroylglutamic acid | 60 |
| Pteroyltriglutamic acid | 400 |
| $N^{10}$-methyl-pteroylglutamic acid | 300 |
| Sulfonamide analog of aminopterin‡ | 400 |
| Pteroylglutamic acid γ-N,N-diethylamide† | 200 |
| Pteroylaspartic acid (d) | 30 |
| Pteroylaspartic acid (racemic) | 30 |
| 4-Amino-pteroyl alanine | 20 |
| *Glutamic acid derivatives* | |
| N-(4-aminobenzoyl)-1(+)-glutamic acid | 100 |
| N-(4-aminobenzenesulfonyl)-1(+)-glutamic acid | 500 |
| *Lumazines* | |
| Lumazine | 250 |
| Dimethyl lumazine | 325 |
| Diphenyl lumazine | 50 |
| *Purines* | |
| Adenine | 200 |
| Guanine | 500 |
| Xanthine | 500 |
| Hypoxanthine | 500 |
| 2,6-Diamino-7-methylpurine | 250 |
| *Quinazolines* | |
| 2,4-Diaminoquinazoline | 65 |
| 2-Methyl-4-hydroxyquinazoline | 300 |
| *Quinoxalines* | |
| 2,3-Dihydroxyquinoxaline | 70 |
| 2,3-Dichloroquinoxaline | 30 |
| *Triazines* | |
| 4,6-Diamino-s-triazin-2-ol (Cyanurodiamide) | 150 |
| 2,4-Diamino-6-(4-dicarboxymethylenethioarsenosoanilino)-s-triazine | 30 |
| *Triazoles* | |
| Benzotriazole | 300 |
| 7-Amino-1-V-triazolo-(d)-pyrimidine | 50 |
| 5-Amino-7-hydroxy-1-V-triazolo (d) pyrimidine | 125 |

* This material is crude and nothing is yet known about the nature of the impurities.
† This compound is a crude product (analyzing about 11.3% pure).
‡ This compound is grossly impure.

transmitted leukemia are affected by this type of treatment.[2,3] A strain previously proven to be influenced by this type of therapy is, therefore, essential to such a screening program. The sole diet of the mice during the experiment consisted of Purina Laboratory Chow of an unknown, but presumably fairly constant pteroylglutamic acid content. No further measures for controlling the intake of the vitamin were attempted. Since the activity of various derivatives as anti-metabolites vary markedly,[7] it is quite possible, therefore, that if the anti-leukemic effect is related to the anti-folic activity, certain less effective antagonists of pteroylglutamic acid may have been missed by this lack of dietary control.

All compounds in these studies which showed a definite chemotherapeutic effect were related in that there were amino groups in the 2 and 4 positions of the pteridine ring or in the analogous configuration in 2,6-diaminopurine. The importance of this particular amino substitution of the pteridine nucleus has been demonstrated by Daniel in her studies on the anti-bacterial action of the pteridines.[8] Hitchings reported that, in studies of a large number of pyrimidines, an

[7] Smith, J. M., Jr., Cosulich, D. B., Hultquist, M. E., and Seegar, D. R., *Tr. New York Acad. Sc.*, 1948, **10**, 82.

[8] Daniel, L. J., Norris, L. C., Scott, M. L., and Heuser, G. F., *J. Biol. Chem.*, 1947, **169**, 689.

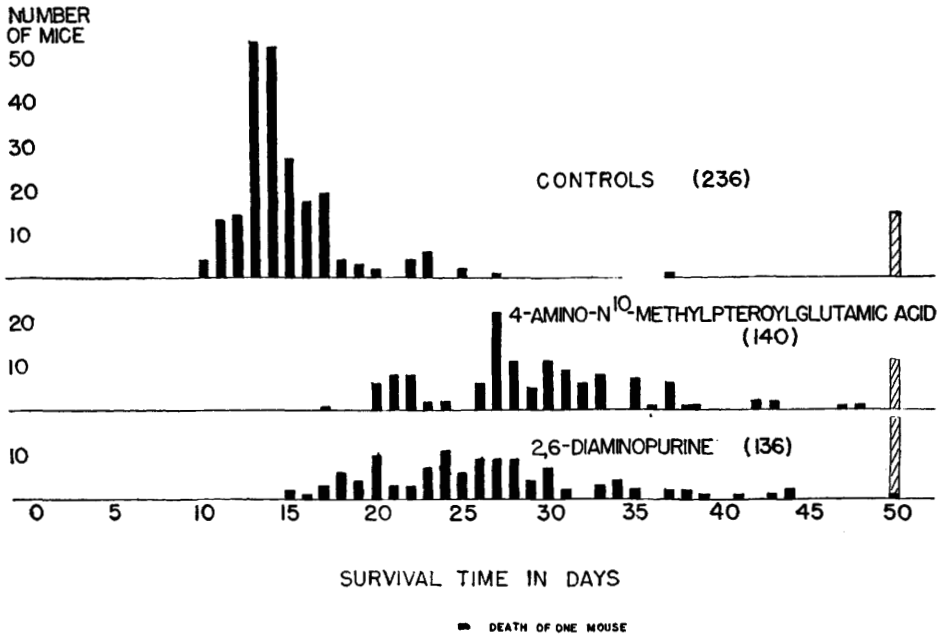


FIG. 1.

inhibition of growth of *L. casei* with PGA in the absence of purine was a property of nearly all 2,4-diamino-pyrimidines and their condensed systems.[9]

Despite the fact that certain simple pyrimidines[10] and pteridines[8] are antagonists of PGA in the metabolism of bacteria, it is of interest to note that all such compounds which were tested against Ak 4 leukemia were without definite effect. The addition of a para amino-benzoic acid moiety to the 2,4-diamino pteridines in 4-aminopteroic acid (2,4-diamino-6-p-carboxyanilinomethylpteridine) and in 4-amino-$N^{10}$-methyl-pteroic acid (2,4-diamino-6 - N-methyl-p-carboxyanilinomethylpteridine) did not increase activity. With the exception of 2,6-diaminopurine, the 2,4-diamino configuration of the pyrimidine ring was effective only when it was a portion of a larger molecule consisting of a pteridine, a para amino-benzoic acid, and an $\alpha$-amino acid. 4-amino derivatives containing the $\alpha$-amino acids, glutamic, aspartic or threonine, possessed chemotherapeutic activity, but 4-aminopteroyl alanine was inactive.

*Summary.* 1. Ninety compounds related to pteroylglutamic acid have been tested for chemotherapeutic effect against transmitted leukemia Ak 4 in mice.

2. Eighty-two of these compounds showed no chemotherapeutic effect by this particular technic.

3. Four showed slight to moderate effect.

4. Four compounds, 4-amino-$N^{10}$-methyl-pteroylglutamic acid, 4-amino-9-methyl-pteroylglutamic acid, 4-amino-9,10-dimethyl-pteroylglutamic acid, and 2,6-diaminopurine have definite chemotherapeutic activity as demonstrated by approximately doubling the average survival time of the mice treated with these compounds.

5. The occurrence of an amino substitution in the second and fourth positions of the pyrimidine ring in all these active compounds has been noted.

We wish to acknowledge at this time the generosity of the following groups in supplying the compounds used in these studies: Dr. C. K. Cain, Department of Chemistry, Cornell University; Calco Chemical Company; Commercial Solvents;

[9] Hitchings, G. H., Elion, G. B., Vander Werff, H., and Falco, E. A., *J. Biol. Chem.*, 1948, **174**, 765.

[10] Hitchings, G. H., Falco, E. A., Sherwood, M. B., *Science*, 1945, **102**, 251.

Eastman Kodak Company; Goodrich Chemical Company; Lederle Laboratories; Merck and Company; National Research Council; Parke Davis and Company; Protein Chemistry Department, Sloan-Kettering Institute; Schwarz Laboratories, Inc.; Southern Research Institute.

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## 17200. A Readily Soluble form of P. B. P. for Use as a Routine Diagnostic Test.

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We reported the preparation of a purified protein antigen from *Brucella,*[1] made by following a modification of Seibert's method for the preparation of PPD (purified protein derivative from tuberculin). This protein substance was a fine, light brown powder of fairly constant chemical composition, not completely soluble in water but easily dissolved by the addition of a few drops of 0.1 N alkali. The solution could be neutralized with 0.1 N HCl and remained clear. This preparation was used by us, as well as by other investigators, for the study of cutaneous hypersensitiveness to *Brucella.* As workers may not have laboratory facilities, we have devised a method whereby the PBP (purified brucella protein) is put up in vial form and in just the right amount for skin testing. By adding a measured quantity of sterile saline buffered solution, the PBP is ready for use.

To prepare the PBP in this way, a weighed amount of the protein antigen is dissolved, as indicated above, to make a concentration of one mg per cc. This solution is dialized through cellulose tubing (Visking Corp.) in cold water for 24 hours. Any impurity that may separate is removed by centrifugation. Then 50 mg of Beta lactose per cc of the PBP solution are dissolved. The resulting liquid should be completely clear. One half cc of this solution, containing 0.5 mg of the *Brucella* protein extract, are bottled in glass vials (about 8 ml capacity) of the type ordinarily used for vaccines, and the contents dried by lyophilization. The lactose has no deleterious effect on the PBP; does not influence the allergic reaction, and is used only to give bulk to the product after drying, since the amount deposited in each vial is infinitesimal.

For skin testing, 5 ml of the sterile diluting fluid are added to each vial, by means of a sterile syringe and needle through rubber stoppers of the vial, and shaken for 2 or 3 minutes to dissolve their contents. The diluting fluid is the same used for Seibert's PPD[2] and is a buffered saline liquid made from the following solutions:

$KH_2PO_4$: 9.078 g dissolved in 1000 cc of distilled water

$Na_2HPO_4 \cdot 2H_2O$: 11.876 g dissolved in 1000 cc of distilled water

Two parts of the solution of the potassium salt are mixed with 8 of the sodium salt, and 0.25% phenol is added.

Each vial contains enough PBP for 50 tests. The tests are performed by injecting 0.1 ml of the diluted protein product with a tuberculin type syringe and a 26-gauge needle into the skin over the flexor surface of the forearm. The site of the injection is examined 48 hours later. Subjects who react positively show a pronounced erythema and edematous induration. Negative cases show no changes.

This preparation was tested on 25 individuals supposed to have been in contact with infected material. Two of them were veterinarians and 2, their assistants. The rest were milkers and dairy workers. Of this group 8 gave positive and 17, negative.

[1] Morales-Otero, P., and González, Luis M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 703.

[2] Seibert, F. B., *et al., Am. Rev. Tuberculosis,* 1934, **30**, 707.