distinguishable from that of the normal beta cells of the islets of normal rabbits. Still other cells present a patchy granularity of the same type and of varying extent, so that all stages of transition from the nongranular cells to cells with a full complement of granular cytoplasm are observed. It is possible that such a transition from nongranular to granular cells of beta type may actually occur under the influence of insulin therapy. This, together with the appearance of proliferation of both of these types of cells, suggests the possibility of improvement or even complete restoration of islet function in relation to carbohydrate metabolism by suitable manipulation of insulin therapy in alloxan diabetes in the rabbit.

Summary. Moderate to extreme hydropic degeneration of the pancreatic ductules and islets was observed in rabbits rendered diabetic by alloxan when the diabetic state had persisted for several months. Such changes have not hitherto been described in alloxan diabetes in the rabbit. The earliest appearance of these alterations was at the end of 45 days after the injection of alloxan and hydropic changes were never absent after 90 days providing that the average fasting blood sugar level during the experiment had been 303 mg per 100 cc, or higher. The hydropic state of the ductules and islets persisted without histological evidence of any further change in association with more or less severe diabetes lasting for periods up to one year. The alpha cells of the islets remained histologically normal but appeared to be increased in number. Preliminary observations demonstrated that the hydropic degeneration of both ductules and islets is reversible by adequate treatment of the diabetic state with insulin. The reversed islet cells appeared to be unduly numerous suggesting proliferation. They were made up in part of indifferent nongranular cells and in part of cells exhibiting varying degrees of granularity of the cytoplasm of beta type. Those with a full complement of granular cytoplasm were indistinguishable in appearance from the beta cells of the islets of Langerhans in the normal rabbit.

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Antibacterial Action of N-Alkyl *p*-Aminobenzoic Acid Derivatives.*

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Yeomans *et al.*,[1] have recently demonstrated the beneficial therapeutic effect of *p*-aminobenzoic acid in louse-borne typhus fever, and several investigators since then have shown its effectiveness in experimental rickettsial infections.[2-5] In a study on the mode of action of sulfonamides, Wyss, Rubin and Strandskov[6] synthesized several ring-substituted *p*-aminobenzoic acid derivatives and tested them for antibacterial action. They found that the 2-Cl, 3-Cl, 2-NH_2, 3-NH_2, and 3-F derivatives of *p*-aminobenzoic acid displayed varying degrees of bacteriostatic action which could be reversed by the addi-

* The authors wish to express their appreciation to Dr. A. R. Surrey for making available the compounds used in this study. His methods of synthesis appear elsewhere.[10] We are also indebted to Mrs. Beatrice Bass for technical assistance.

1 Yeomans, A., Snyder, J. C., Murray, E. S., Zarafonetis, C. J. D., and Ecke, R. S., *J. A. M. A.*, 1944, **126**, 349.

2 Anigstein, L., and Bader, M. N., *Science*, 1945, **101**, 591.

3 Hamilton, H. L., Plotz, H., and Smadel, J. E., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 255.

4 Hamilton, H. L., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 220.

5 Murray, E. S., Zarafonetis, C. J. D., and Snyder, J. C., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 80.

6 Wyss, O., Rubin, M., and Strandskov, F. B., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 155.

tion of *p*-aminobenzoic acid itself. At pH 7.0 the most active derivative (2-Cl) had a bacteriostatic value equal to sulfapyridine. Hirsch[7] found *p*-aminobenzamide to be as active as sulfanilamide, and postulated that a new group of substances with chemotherapeutic effects toward bacterial infections might be found among the derivatives of *p*-aminobenzamide.

The present authors investigated the effects upon sulfonamides of a group of N-substituted *p*-aminobenzoic acid derivatives and local anesthetics derived from *p*-aminobenzoic acid.[8,9] During these studies it was noted that several of the compounds, notably the methyl *p*-alkylaminobenzoates, gave evidence of possessing considerable antibacterial activity. It seemed appropriate, therefore, to study more completely the general antibacterial properties of the various alkyl-substituted *p*-aminobenzoic acids.

Methods of Antibacterial Tests. The medium employed throughout these tests was trypticase soy broth (B.B.L.). This medium was found to support excellent growth of all test organisms without the addition of serum or other growth-promoting factors. The organisms employed were as follows: Pneumococcus type II, *Streptococcus pyogenes* (C203), *Staphylococcus aureus, Haemophilus ducreyi, Pasteurella pestis, Eberthella typhi, Vibrio cholerae,* and *Mycobacterium avium.*

A series of dilutions of the *p*-aminobenzoic acid derivatives ranging from 1:1000 to 1:64,000 was prepared directly in the broth. Owing to the relative insolubility of several of the compounds in water, the initial dilutions were made by dissolving the compounds in a small amount of alcohol followed by the addition of broth. Controls containing an equivalent amount of alcohol had no visible effect upon the growth of the organisms. Tubes containing 5 cc of each dilution were autoclaved at 10 lb for 10 minutes, and after cooling 0.05 cc of a 1:100 dilution of a 24-hour broth culture of test organism was added to each series. The tubes were incubated at 37°C and examined after 24 hours for visible growth (48 hours in tests employing the pneumococcus, streptococcus and *P. pestis*). Bacteriostasis was recorded for those tubes showing no growth or growth less than half that of the drug-free broth control at the initial observation time. All tubes containing no growth after 72 hours at 37°C were subcultured by transferring 3 4-mm loopfuls to 10 cc of fresh trypticase soy broth. Failure of growth to appear in the subculture tube after 72 hours' incubation was taken as evidence of a bactericidal action by the drug in the original medication tube. Results of these tests are presented in Table I.

Results. It is apparent that the antibacterial activity of *p*-aminobenzoic acid is slightly increased by the substitution of an ethyl or butyl group in the N-position. In the series of methyl *p*-alkylaminobenzoates and ethyl *p*-alkylaminobenzoates maximum activity is found in the N-propyl-substituted compounds. In this case the N-ethyl and N-*n*-butyl derivatives are about equal in action which is somewhat less than the N-propyl compounds. The series of benzamides display a direct correlation between chain length of the N-substituent and degree of antibacterial action. The lowest degree of activity is exhibited by the ethyl derivative with proportionately increasing activity being found in the propyl and *n*-butyl derivatives.

Antagonism by p-aminobenzoic acid. Inasmuch as Hirsch[7] noted that *p*-aminobenzamide was antagonized by the presence of *p*-aminobenzoic acid, and we showed that the *p*-alkylaminobenzamides did not antagonize sulfonamides[8] it appeared worthwhile to study the effect of *p*-aminobenzoic acid upon the *p*-alkylaminobenzamides, and upon diethylaminoethyl - *p-n* - butylaminobenzoate hydrochloride (the *n*-butyl derivative of procaine hydrochloride, U.S.P.). The latter compound had also been found to have none of the sulfonamide antagonizing properties of procaine itself.[9]

Accordingly, diethylaminoethyl-*p-n*-butyl-

[7] Hirsch, J., *Science,* 1942, **96**, 139.

[8] Goetchius, G. R., and Lawrence, C. A., *J. Bact.,* 1944, **48**, 683.

[9] Lawrence, C. A., and Goetchius, G. R., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 180.

[10] Surrey, A. R., and Hammer, H. F., *J. Am. Chem. Soc.,* 1944, **66**, 2127.

TABLE I. Antibacterial Action of *p*-Aminobenzoic Acid Derivatives.

Figures in each left-hand column represent highest dilution × 1000 exhibiting bacteriostatic action, *i.e.*, 2 = 1:2000, etc. Figures preceded by symbol < denotes highest concentration tested (1:1,000) failed to show evidence of bacteriostasis. Figures followed by asterisk (*) represents highest dilution exhibiting bactericidal activity.

Compound	Pneumococcus type II	*Streptococcus pyogenes*	*Staphylococcus aureus*	*Haemophilus ducreyi*	*Pasteurella pestis*	*Eberthella typhi*	*Vibrio cholerae*	*Mycobacterium avium*
p-Aminobenzoic acid	<1	<1	<1	1	1	<1	<1	<1
p-Ethylaminobenzoic acid	2 1*	<1	<1	1	2	<1	<1	<1
p-Butylaminobenzoic acid	2 1*	1	<1	1	4 2*	<1	2 1*	<1
Diethylamino ethyl *p*-ethylamino benzoate. HCl	4	<1	<1	<1	<1	<1	<1	<1
Diethylamino ethyl-*p*-*n*-butylamino benzoate. HCl	8 4*	4 4*	1 1*	2 2*	2 2*	1	2 1*	1
Methyl *p*-ethylamino benzoate	2	1	<1	1	2	<1	<1	<1
Methyl *p*-propylamino benzoate	8 1*	16 8*	<1	8 2*	8 8*	<1	4 1*	<1
Methyl *p*-*n*-butylamino benzoate	2 1*	1	<1	2	2	<1	<1	<1
Ethyl *p*-ethylamino benzoate	16 1*	4 1*	<1	4	8 8*	<1	4 1*	<1
Ethyl *p*-propylamino benzoate	32 1*	16	<1	4 1*	16 2*	1	2	<1
Ethyl *p*-n-butylamino benzoate	16	4	<1	32 1*	4 2*	1	1	1
p-Ethylamino benzamide	2	<1	<1	1	1	<1	<1	<1
p-propylamino benzamide	4	2	<1	2	1 1*	1	1 1*	<1
p-*n*-Butylamino benzamide	8 2*	4 2*	1	4 1*	2 2*	1 1*	2 1*	1

aminobenzoate hydrochloride and *p*-*n*-butylaminobenzamide were dissolved in 1% concentration directly into trypticase soy broth containing 10^{-3}, 2×10^{-4}, 10^{-4}, and 2×10^{-5} dilutions of *p*-aminobenzoic acid. The initial concentrations of the 2 test compounds were then diluted serially up to and including dilutions of 1:12,800. The compounds were also diluted in a similar manner in trypticase soy broth containing no *p*-aminobenzoic acid for control titrations. The tubes were autoclaved at 10 pounds for 10 minutes, and after cooling were inoculated with a 4-mm loopful of a 24-hour broth culture of *Streptococcus pyogenes* (C203). The inoculated tubes were incubated as before and the bacteriostatic and bactericidal end-points recorded.

Results. The data obtained in this study revealed that *p*-aminobenzoic acid does not antagonize the antistreptococcal activity of either diethylaminoethyl-*p*-*n*-butylaminobenzoate hydrochloride or *p*-*n*-butylaminobenzamide. It would appear from these findings, therefore, that N-alkylation of these *p*-aminobenzoic acid derivatives not only nullifies their antagonistic action upon sulfonamides, but also gives their own antibacterial activity an "immunity" to antagonism by *p*-aminobenzoic acid.

Summary. 1. The bacteriostatic and bactericidal activity of a series of N-substituted *p*-aminobenzoic acid derivatives have been determined.

2. Among the acids the order of decreasing activity is as follows: *p*-butylaminobenzoic acid > *p*-ethylaminobenzoic acid > *p*-aminobenzoic acid.

3. Of 2 procaine hydrochloride (U.S.P.) derivatives, the N-butyl derivative displays greater antibacterial action than the N-ethyl.

4. Among the methyl and ethyl esters of *p*-aminobenzoic acid, the N-propyl derivatives display more activity than the N-ethyl or N-butyl compounds. The latter 2 were approximately equal in respect to antibacterial action.

5. The order of decreasing activity among the *p*-alkylaminobenzamides was: butyl > propyl > ethyl.

6. Diethylaminoethyl-*p-n*-butylaminobenzate hydrochloride and *p-n*-butylaminobenzamide were not antagonized by the presence of *p*-aminobenzoic acid when tested in trypticase soy broth against *Streptococcus pyogenes* (C203).

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Action of Certain Carbohydrates on the Reaction of *Eberthella typhosa* with Antibody O.

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In a previous publication, Olitzki, Shelubsky and Hestrin[1] reported that certain carbohydrates promote the pathogenicity of *E. typhosa*. Since Gram-negative microorganisms are normally eliminated from the peritoneal cavity in the mouse mainly by bacteriolysis a short time after the onset of infection,[2] the possibility that the carbohydrates, which alter pathogenicity might also interfere with a bacteria-antibody reaction seemed to deserve consideration. The influence of the carbohydrates previously employed in tests of pathogenicity on the agglutination of typhoid strain 0901 by its O-antibody, the presence of which in antisera parallel the bactericidal activity,[3] has accordingly been examined. The results of the agglutination tests are shown in Table I.

Table I shows that the carbohydrates used for testing, *viz.* dextran, levan, mucin and pectin, markedly inhibit agglutination. The results were the same whether bacteria were added to the serum-carbohydrate mixture immediately after its preparation or after it had been incubated for 4 hours at 37°C.

The mechanism of the inhibition could be a serological reaction between the test carbohydrates and the typhoid antibody. We have not, however, been able to detect a visible precipitation reaction when the carbohydrates were mixed with anti-typhoid serum. In order to ascertain whether the inhibition is produced by interference on the part of the carbohydrates with fixation of antibodies by the bacterium, or by an intervention in the second stage of the agglutination reaction, we measured the effect of the more active of the inhibitors (dextran 2%, levan 2%, pectin 2%, and mucin 5%) on antibody-nitrogen uptake.

The carbohydrate solutions were sharply centrifuged to remove all insoluble particles. Heat-killed and washed bacteria sediment containing 0.419 mg nitrogen was then suspended in 2.0 cc of the solutions. After the selected carbohydrates and bacteria had been in contact for 2 hours at 37°C, the immune serum was added and the mixture allowed to stay for 24 hours in the ice box. The bacteria were then removed and washed and the N-uptake determined by a Micro-Kjeldahl method. In another series of experiments the serum was left in contact with an inhibitor for 2 hours at 37°C. Examination of this mixture failed to reveal any visible precipitation. Bacterial sediment containing 0.260 mg nitrogen was then suspended in the mixture. The suspension was left for 24 hours in the ice box. The bacteria were then removed and examined as in the previous series. Both series of experiments gave similar results. The control test without the addition of inhibitor showed that the bacteria are normally able to remove in 24 hours 0.033 mg N from 0.1 ml serum and 0.061 mg N from 0.2 ml serum. In the pres-

[1] Olitzki, L., Shelubsky, M., and Hestrin, S., in press.

[2] Olitzki, L., and Koch, P. K., *J. Immunol.*, 1945, **50**, 229.

[3] Felix, A., and Olitzki, L., *J. Immunol.*, 1926, **11**, 31.