in part represent interconvertible species. Tiselius, Hjerten and Levin have obtained three components by chromatography.[33] Hughes[34] found that serum albumin does not contain a full equivalent of SH on the basis of a molecular weight of 68,000, but that a fraction can be separated which does show a full equivalent of SH. At a *p*H under 4 boundary spreading is observed in the ultracentrifuge[29] but this may be due to reversible denaturation.

In addition to this incomplete bibliography on the inhomogeneity, work now in progress in this Laboratory with an entirely different system recently discovered and soon to be reported, has shown that both human and bovine serum albumin can be resolved by C.C.D. into several components. Even artificial mixtures of the two can be separated with the separate components of each still retaining their respective separate partition positions. Thus while the TCA systems are not suited to the resolution of the very closely related components of serum albumin they do clearly separate several other protein components as Figs. 4, 6 and 7 show.

The run which gave Fig. 6 was made on a fraction obtained electrophoretically from serum. It had the green color of bilirubin and was known to have a certain amount of α_1-globulin in it. At the start of the run a moderate amount of insoluble material separated at the interface and was discarded. Three bands aside from the albumin one are obvious. One of them is the α_1-globulin. Recovery of the albumin band gave a white solid without the green color of the bilirubin but in about 72% of the amount expected, had the same original weight of Cohn V fraction been taken.

We wish to thank Drs. Henry Kunkel and R. Trautman for helpful discussions as well as for samples of albumin. We also thank Mr. D. Rigakos for the elementary analyses.

NEW YORK, N. Y.

(33) A. Tiselius, S. Hjerten and O. Levin, *Arch. Biochem. Biophys.*, **65**, 132 (1956).

(34) W. L. Hughes, Jr., THIS JOURNAL, **69**, 1836 (1947).

[CONTRIBUTION FROM THE INSTITUTE OF MICROBIOLOGY, RUTGERS, THE STATE UNIVERSITY]

Immunochemical Study of a Bacterial DNA[1]

BY JOHN H. PHILLIPS,[2] WERNER BRAUN AND OTTO J. PLESCIA

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Evidence has been obtained that DNA contributes to the antigenic specificity of a 0.5% phenol-extracted "deproteinized" bacterial DNA preparation. It is based primarily upon the appearance of a Feulgen-positive zone among several bands of precipitation in gel-diffusion tests, the disappearance of antigenic components from the DNA preparation following DNAase treatment as shown by quantitative precipitin analysis, and the occurrence of cross-reactions of DNA from calf thymus and salmon sperm with antiserum produced against bacterial DNA. Preliminary evidence suggests that the extent of these cross-reactions increases with limited depolymerization of the antigenic material by DNAase.

Introduction

A number of earlier studies[3-6] have suggested a serological reactivity for deoxyribonucleic acid (DNA), but the supporting evidence has been indirect and disputable. For example, the precipitations observed by Lackman, *et al.*,[3] could be ascribed to charge effects rather than antigen–antibody reactions, since they proved sensitive to *p*H and ionic strength in the range in which the precipitin reaction is stable. Similarly, the studies by Blix, *et al.*,[4] failed to reveal whether their complement-fixing antibodies were specific for DNA or some other unrelated antigen present. Also, the indirect method employed is questionable when either the antigen or antibody is anticomplementary, as was found to be the case. Medawar's[5] observation regarding effects of DNAase upon a factor controlling tissue transplantability, though strongly suggestive of DNA antigenicity, has not lent itself to direct quantitative measurements. In view of the potential significance of the availability of antibodies specifically directed against DNA or the DNA moiety of a nucleoprotein, and the availability of a different procedure for the isolation from bacterial cells[7,8] of a highly polymerized DNA with transforming activity, the problem has been reinvestigated. The results of these studies, reported here, strongly indicate the existence of an antigenic specificity associated with DNA and the availability of procedures which should lend themselves to further quantitative analyses of this phenomenon.

Experimental

Preparation of Immunizing Antigens.—The material was obtained from cells of the bacterium *Brucella abortus*, strain 19, by extraction with 0.5% phenol in citrate-saline at 37° for 48 hr. and subsequent deproteinization by repeated treatments (2–5 times) with chloroform and amyl alcohol.[8,9] The resulting material appeared to consist principally of DNA on the basis of its absorption at 260 mμ in saline, its viscosity, its susceptibility to DNAase action, a positive Dische–Stumpf reaction and its purine and pyrimidine content as shown by chromatography following hydrolysis. However, on the basis of the biuret test and N/P ratio it was estimated to contain approximately 25% protein which could not be dissociated by repeated treatments with chloroform–amyl alcohol.[8]

(1) These studies were aided in part by grants from the U. S. Public Health Service (E-1137) and the National Science Foundation (G-2184).

(2) Waksman–Merck Postdoctoral Fellow.

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(4) U. Blix, C. N. Iland and M. Stacey, *Brit. J. Exptl. Pathol.*, **35**, 241 (1954).

(5) P. B. Medawar, Oral reports, 1957.

(6) H. Masamune and S. Tsuiki, *Tohoku J. Exptl. Med.*, **61**, 171 (1955).

(7) W. Braun and J. Whallon, *Proc. Natl. Acad. Sci. U. S.*, **40**, 162 (1954).

(8) W. Braun, J. Whallon and J. Phillips, *Nature*, **180**, 1356 (1957).

(9) M. Sevag, D. Lackman and J. Smolens, *J. Biol. Chem.*, **124**, 425 (1938).

Preparation of Antisera.—New Zealand white rabbits (5–6 lb.) were immunized by intravenous injections of a saline solution of DNA from either smooth or mucoid *B. abortus* cells or by the injection of whole formalin-killed cells suspended in saline. The total quantity of DNA administered to each rabbit was 1.7 mg., either as extracted DNA or contained in whole cells, measured by the Dische–Stumpf reaction. The injection dose was increased progressively in the course of 12 injections from 0.05 to 0.25 mg. administered at regular intervals during one month. Lower dose levels were found to be less effective. The animals were bled from the heart one week after the last injection. The serum was stored at −10°.

Precipitin Analyses.—Gel-diffusion studies were carried out according to the technique of Ouchterlony[10]; various concentrations of antigen were used but the optimum concentration was found to be 0.5 mg. DNA/ml. Antisera were used undiluted. Quantitative precipitin tests followed essentially the procedures of Heidelberger and Kendall,[11] except that the precipitates were dissolved in 0.25 *M* acetic acid and the amounts measured spectrophotometrically at 277 mμ, the wave length of maximum absorption for the dissolved precipitates.

Preparation of Test Antigens.—The concentration of test antigens was adjusted by Dische–Stumpf reactions.[12] Additional deproteinization of the test antigens was attempted by a variety of methods. Trypsin-treated DNA was prepared by incubating a mixture of 0.055% DNA (0.55 mg. per ml. on the basis of Dische–Stumpf reaction) and 0.03% trypsin (salt-free 2 × crystallized, Worthington TR 540) at *p*H 7.0 for 1.5 hr. at 37°. Chymotrypsin (Armour Lot 283 crystalline salt-free) in conjunction with trypsin was used at concentrations of 0.03% for each enzyme. Deproteinization by salt treatment was attempted by suspending the antigen in saturated sodium chloride solution for one month at 5°; the supernatant was subsequently precipitated with 95% ethyl alcohol, washed with 70% ethyl alcohol and redissolved in saline. A combined detergent–salt treatment consisted of making a saline solution containing 0.055% DNA and 0.025% sodium lauryl sulfate and letting it stand at 5° for 24 hr.; this was followed by increasing the salt concentration to molarity, centrifuging, precipitating with 95% ethanol and redissolving in saline. Treatment with DNAase (Worthington 1 × crystallized) was carried out by the addition of DNAase (final concentration 0.03%) to a 0.05% solution of DNA in saline containing 0.0009 *M* $MgSO_4$. All antigens for the gel-diffusion tests were preserved by the addition of sodium methiolate to a concentration of 0.01% and stored at 5°.

Preparation of DNA from Other Sources.—Calf thymus and salmon sperm DNAs also were used as test antigens. Calf thymus was extracted with Duponol[13] and with 0.5% phenol after treatment of homogenized tissues with 6 *M* NaCl. A sample of salmon sperm DNA prepared by 0.5% phenol extraction was furnished by E. R. Squibb and Sons.

Results

Gel-diffusion Tests.—The antisera first were tested for precipitating antibodies against the homologous Brucella DNA by the gel-diffusion method.[10] Within 72 hr. five clearly distinguishable bands of precipitation developed, indicating more than one antigen–antibody system. Three of these bands can be seen in a tracing of the photographic reproduction, shown in Fig. 1. The band closest to the antigen well was observed to split into three bands when the concentration of antigen was decreased. This splitting could not be reproduced photographically. No significant alterations of these reactions could be detected following treatment of the test antigen with either trypsin, chymotrypsin + trypsin, saturated NaCl or detergent. Also, absorption of the antisera with formalin-killed whole cells, or with insoluble cellular residue remaining after phenol extraction, failed to alter these results.

After treatment of the test antigen with DNAase, however, a noticeable decrease in intensity of one of the bands and a simultaneous increase in another took place. DNAase alone showed no reaction with the antisera. The possible significance of this shift will be discussed subsequently together with the results of the quantitative precipitin tests.

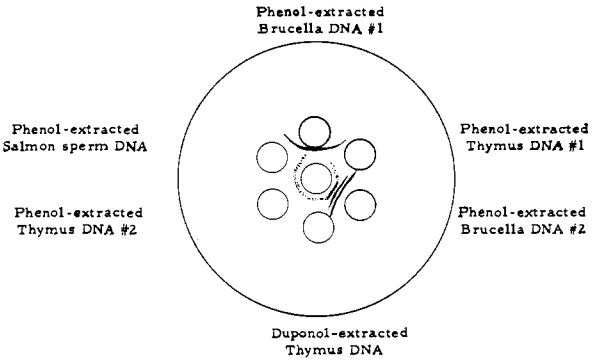

Fig. 1.—Gel-diffusion analysis of rabbit antiserum to Brucella DNA. The antiserum was placed in the center well surrounded by different antigen preparations in the outer wells. The notations #1 and #2 refer to different batches of antigens obtained by varying slightly the extraction procedure. Shown here is a tracing of the photographic reproduction; the solid lines are definite pronounced bands of precipitate, but the stippled lines denote weak diffuse precipitation.

The presence of DNA in at least one of these precipitin reactions was indicated by the results of the Feulgen test applied to developed gel-diffusion plates in the following manner: The agar gels were washed free of soluble materials by at least four cycles of flooding the plates with chilled saline and decanting after 24 hr., during which time the plates were kept at 5°. After washing, the plates were treated with 1 *N* HCl at 50° for 5 minutes and, after the removal of the acid, Schiff reagent was applied and replaced by saline after 2 hr. In numerous trials with both untreated and deproteinized antigens one zone of precipitation (the band closest to the antiserum well in Fig. 1) consistently yielded a strong positive reaction suggesting the presence of DNA in the precipitate. The absence of detectable reactions for the other zones of precipitation does not necessarily indicate the absence of DNA in these precipitates but may merely reflect that the amount of DNA present is too small for detection by this procedure.

Quantitative Precipitin Reactions.—A more decisive indication of the involvement of DNA in the precipitin reactions just described was obtained by quantitative procedures in which various amounts of antigen were mixed in test-tubes with a constant amount of antiserum.

A solution of 47.6 mg. DNA per 100 ml. of 0.9% saline was freed of insoluble material by centrifugation at 5° for 1 hr. at 3100 × *g* and filtration through Whatman #2 paper. Duplicate aliquots varying from 0.5 to 2.5 ml. were mixed with

(10) O. Ouchterlony, *Arkiv. Kemi. Mineral. ch Geol.*, **26B**, 1 (1948).

(11) M. Heidelberger and F. Kendall, *J. Exptl. Med.*, **61**, 559 (1935).

(12) P. K. Stumpf, *J. Biol. Chem.*, **169**, 367 (1947).

(13) E. R. M. Kay, N. S. Simmons and A. L. Dounce, THIS JOURNAL, **74**, 1724 (1952).

0.5 ml. of centrifuged pooled rabbit antiserum + 0.5 ml. of 0.03 *M* EDTA in saline and brought to a final volume of 4.5 ml. with 0.9% saline. The EDTA was added because it was used subsequently to chelate Mg required by the DNAase. Mixtures were then incubated at 37° for 2 hr., followed by 48 hr. at 5°. An identical set of aliquots was used for the preparation of normal rabbit serum controls; serum and antigen blanks were also prepared.

The remaining 86 ml. of DNA solution was treated with DNAase at 37° by adding 21.6 ml. of a previously centrifuged solution containing 20 mg. of DNAase in 24 ml. of 0.1 *M* $MgSO_4$. Aliquots were removed for mixing with antiserum at various intervals and DNAase activity was terminated by the above described addition of EDTA. Again normal serum controls and antigen blanks were prepared for each time period sampled (both proved to be identical). Precipitates were centrifuged and washed according to established procedures[11] and were finally dissolved in 0.25 *M* acetic acid. The amounts of precipitate, measured in terms of absorption at 277 mμ, were plotted against antigen concentrations in terms of DNA after subtracting the values obtained for the normal serum–antigen controls. The data are given in Table I, and the quantitative precipitin curves are shown in Fig. 2.

and its content of DNA decreased to about 9% after treatment with DNAase. This fact together with decreases in the extent of precipitation indicated the presence of DNA in the precipitates. It is also noteworthy that following prolonged treatment with the enzyme the antigenic activity was virtually abolished. These quantitative precipitin reactions, and their dependence upon DNAase treatment, proved reproducible in subsequent tests. It is well to note here that in early studies of bacterial transformation, the conclusion that DNA was involved was based largely on a similar sensitivity of the reaction to DNAase treatment of the DNA-containing preparation.

For the interpretation of the above observations it was necessary to determine both the possibility of coprecipitation of DNA with an unrelated antigen–antibody precipitate and the extent of contamination of the DNAase by proteolytic enzymes. In appropriate control tests it was found that the antigenic material here used did not coprecipitate in reactions involving either egg albumin–rabbit anti-egg albumin or Type III pneumococcal polysaccharide and its corresponding precipitating antibody. Absence of proteolytic activity in the DNAase was not only claimed by the manufacturer but was indicated also by a lack of liberation of any

TABLE I

PRECIPITATION OF ANTIBODY FROM RABBIT ANTISERUM BY A DNA PREPARATION TREATED WITH DNAase FOR VARIOUS TIMES

DNA + DNAase (ml.)[a]	0.5 ml. rabbit antiserum Period of DNAase treatment (hr.) 0[b]	0.25	1	4	16	64	0.5 ml. normal rabbit serum[d] Period of DNAase treatment (hr). 0[b]	0.25	1	4	16	64
0.5	0.102[c]	0.045	0.049	0.036	0.048	0.033	0.005	(0.000)[e]	0.008	(0.001)	(0.009)	0.009
0.5	.100	.046	.050	.034	.047	.034	.002		.001			.006
1.0	.104	.045	.081	.056	.059	.039	.005	(.000)	.023	(.003)	(.023)	.023
1.0	.102	.046	.084	.055	.063	.036	.006		.020			.020
1.5	.125	.054	.105	.064	.084	.038	.007	.001	.034	.005	.036	.035
1.5	.126	.054	.103	.063	.083	.035	.008	.000	.036	.004	.035	.035
2.0	.102	.061	.114	.072	.111	.054	.010	(.001)	.048	(. 005)	(.047)	.050
2.0	.100	.060	.116	.071	.110	.051	.011		.050			.047
2.5	.097	.086	.159	.060	.103	.075	.018	(.001)	.058	(.007)	(.058)	.063
2.5	.098	.084	.156	.063	.099	.074	.019		.060			.060

[a] Concentration of DNA was 0.476 mg./ml., as determined by the Dische–Stumpf reaction. The concentration of DNAase was 0.167 mg./ml. [b] No DNAase present. [c] The values represent the optical densities at 277 mμ of the solutions of the precipitates in 0.25 *M* acetic acid. [d] Antigen controls, with saline replacing serum, were also included. In each case these were identical to the controls consisting of normal serum plus antigen, indicating essentially no interaction between normal serum and antigen leading to increased precipitation. [e] The values in parentheses are calculated values, based on a linear relationship obtained between the extent of non-specific precipitation and the amount of DNA preparation used.

The number of maxima in the zero time curve again indicates the complexity of the material under study. However, of greatest significance appears to be the finding that significant changes occurred in this curve with increasing time of treatment of the antigen with DNAase. It can be seen that a significant decrease occurred in the amount of precipitate throughout most of the tested range following treatment with DNAase for as little as 15 minutes. The precipitates formed in the region of lowest antigen concentration were then analyzed for the presence of DNA by measurements of the ratio of 260 mμ/277 mμ absorbing materials. This ratio was initially 0.94 and decreased to 0.69 with increasing time of DNAase treatment. On the basis of these ratios it is estimated that the specific precipitate contained initially about 27% DNA amino acids or peptides detectable by paper chromatography in the reaction mixture.[14]

Cross Reactions.—The interactions between the described antisera and DNA isolated from different strains of the same bacterial species or from vertebrates so far have been studied only in gel diffusion tests. Nevertheless, interesting differences were detected by this essentially qualitative procedure.

DNA isolated from smooth *Brucella abortus* cells and tested either against its homologous antiserum or a serum prepared against DNA from mucoid *B. abortus* cells showed essentially identical bands. The same results were obtained in reciprocal tests.

In contrast, calf thymus DNA obtained by extraction with either phenol or Duponol showed only weak precipitin reactions with anti-*B. abortus*

(14) J. Noval, unpublished data.

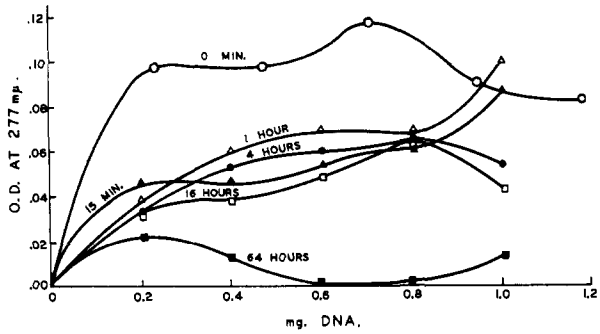

Fig. 2.—Effect on the antigenicity of a Brucella DNA preparation by DNAase treatment. The curves show the extent of precipitation of antibody from 0.5 ml. of a pool of rabbit antiserum as a function of the amount of antigen used and the time of treatment with DNAase. The ordinate is the optical density at 277 mμ which is proportional to the amount of precipitate formed. All points are averages of duplicate determinations.

DNA sera (Fig. 1). Similar weak reactions were displayed by phenol-extracted salmon sperm DNA (Fig. 1). However, after treatment for a short time of the antigens, isolated from vertebrate cells, with DNAase, a marked increase in cross-reactivity occurred. As can be seen in Fig. 3 this involved the appearance of dense zones of precipitation.

Discussion

Evidence in favor of an involvement of DNA in precipitin reactions with antisera against a phenol-extracted bacterial DNA can be derived from the described results of gel-diffusion tests and quantitative precipitin reactions. The detection of a Feulgen-positive zone in gel-diffusion tests, the disappearance from specific precipitates of antigenic components containing DNA after treatment with DNAase and the cross-reactions of DNA from unrelated species with antisera against bacterial DNA, together strongly suggest that DNA itself may contribute to the antigenic specificity of the preparations used.

It is recognized that both in the gel-diffusion tests and in the quantitative precipitin reactions DNA could have been precipitated without itself being antigenic because it might be firmly bound to antigens other than DNA or because it might have coprecipitated with an unrelated antigen–antibody precipitate. However, the virtual abolishment of precipitability of antibody by the DNA preparation after prolonged enzymatic treatment (Fig. 2), the absence of coprecipitation of DNA with known unrelated antigen–antibody precipitates, and the absence of any detectable proteolytic activity in the DNAase used, would favor the conclusion that DNA itself contributed to the specificity and is not merely an inert part of the antigens. These arguments, however, do not eliminate completely an additional possibility that the DNA itself is non-antigenic but serves as a carrier for an antigen that by itself forms only soluble complexes with antibody. Studies designed to test this possibility are now in progress.

The present indication that DNA possesses antigenic specificity is further supported by the apparent interdependence of the specific precipitates formed as a function of the time of treatment with DNAase. First of all, there is a vacillation in the maximum amounts of precipitation in regions of higher antigen concentration (Fig. 2). In addition, it is apparent from the quantitative precipitin curves that there are regions of antigen concentrations where the amount of precipitate formed increases with limited DNAase treatment (see 15 minute and 1 hr. curves). This may be explained either on the basis of the formation of new antigens, corresponding possibly to antigens also formed *in vivo*, or more of the previously existing antigens. Also, there is a tendency for points of maximum precipitation to shift into regions of higher antigen concentration. This would be consistent

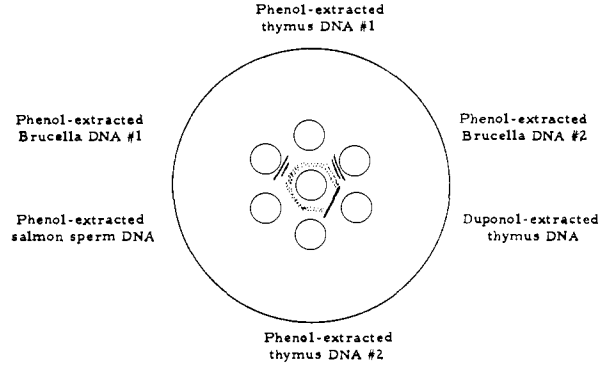

Fig. 3.—Effect of DNAase treatment on the antigenicity of calf thymus, salmon sperm and Brucella DNA preparations, as revealed by gel-diffusion analysis. The results are to be compared with those shown in Fig. 1, obtained with the same DNA preparations but without DNAase treatment. Shown here is a tracing of the photographic reproduction; the solid lines are definite pronounced bands of precipitate, but the stippled lines denote weak diffuse precipitation.

with the assumption that the breakdown products are less effective in precipitating antibody, requiring therefore an increased concentration. These changes in the precipitin curves are consistent with the shift in the gel-diffusion pattern following DNAase treatment, as pointed out already. To get more decisive information on the antigenic activity of such breakdown products, studies utilizing radioactively labeled DNA would be desirable. Also, more refined physical-chemical analysis of the antigenic preparation and of the precipitates collected in different ranges of antigen concentration (Fig. 2) might elucidate some of the questions here raised.

Additional evidence in favor of the production of DNA-specific antibodies has come from preliminary tests of the activity of the described antisera in a biological system. It was observed that these antisera were able to antagonize selective effects of DNA plus DNAase upon bacterial population changes. It had been found previously[7] that the addition of DNA plus DNAase to non-smooth bacterial cultures resulted in the selective establishment of spontaneously arising smooth type cells. This effect mediated by DNA breakdown products[15]

(15) W. Braun, W. Firschein and J. Whallon, *Science*, **125**, 445 (1957).

now has been found to disappear in growing Brucella cultures in the presence of antisera prepared against Brucella DNA but not in the presence of normal rabbit serum. It would be most desirable to extend the study of the influence of these antisera to include a variety of biological systems, such as bacterial transformation mediated by highly polymerized DNA. Such studies are planned for the near future.

The present information fails to reveal whether the formation of specific antibodies requires both protein and DNA or involves only the DNA molecule (and some of its breakdown products). However, from a genetic, biological and analytical standpoint, it would appear that the answer to this question may be less critical than the indication that DNA (and some of its breakdown products) may contribute to the specificity of an antigen. It must be determined in future studies to what extent the protein present in phenol-extracted bacterial DNA contributes to the formation of what appear to be DNA-specific antibodies, and whether such protein, if required, must be associated with the DNA in a uniquely stable fashion. Such nucleoprotein stability is indicated by some of the characteristics of the material here used.[8] Heidelberger and co-workers[16] previously have called attention to a bacterial nucleoprotein of unusual stability, which was antigenic and dissociated only by treatment with alkali.

The results here reported would seem to furnish procedures applicable to further studies on quantitative aspects of problems associated with DNA antigenicity. In particular, quantitative precipitin reactions with polymerized and DNAase-treated DNA, extracted by different procedures and from different species, should be studied with homologous and heterologous antisera. If immunochemical techniques can be used reliably to detect differences between DNAs of different species on the basis of the dependence of cross-reactivity upon degree of polymerization, it may become possible to obtain additional information regarding the chemical nature of DNA. Also, if future studies confirm the present evidence for antigenic specificity of DNA, it may become possible to reinvestigate the feasibility of affecting genetic processes that are assumed to be controlled by DNA. However, it is possible that specific alterations by antisera of intracellular "information centers" for hereditary traits may continue to prove difficult due to permeability barriers.

Acknowledgment.—The initation and execution of these studies was greatly aided by the interest and suggestions of Professor M. Heidelberger. For this the authors wish to express their gratitude.

NEW BRUNSWICK, NEW JERSEY

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[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

The Isolation of a Second Antibiotic from *Streptomyces hygroscopicus*

BY ROBERT L. MANN AND W. W. BROMER

RECEIVED OCTOBER 25, 1957

S. hygroscopicus has been shown to produce a second antibiotic substance, hygromycin B. Isolation of hygromycin B was accomplished by chromatography on a cation-exchange resin, carbon adsorption and countercurrent distribution. Properties and partial characterization of the new antibiotic have been described.

The isolation of hygromycin from *S. hygroscopicus* and the determination of its structure have been reported.[1-3] In the early stages of the purification of hygromycin, paper chromatographic evidence was obtained that more than one antibiotic was produced by the organism. This paper is concerned with the isolation and partial characterization of a second antibiotic substance, hygromycin B. This new antibiotic is primarily of importance because of its activity against helminths, including ascarids in swine.[4]

Broths suitable for the isolation of hygromycin B were fermented according to methods described for the production of hygromycin.[1] Antibiotic potency was determined with *Bacillus subtilis* using a 16 hr. paper disc diffusion assay. A crude concentrate of hygromycin B was obtained by removing the antibiotic from the fermentation broth with a cation-exchange resin (Amberlite IRC-50 in the Na^+ cycle) and eluting with 0.1 *N* hydrochloric acid. Further purification was achieved by adjusting the resin eluate to *p*H 10.5 and treating with carbon (Norite S.G.) to adsorb the hygromycin B. The carbon was removed by filtration, washed with water, and the antibiotic was eluted with a solvent composed of 1 part of concentrated ammonium hydroxide, 3 parts of water and 6 parts of acetone. The eluate was concentrated to about one-tenth of the original volume and the hygromycin B was precipitated with acetone. The gummy precipitate was dissolved in methanol. When ether was added, a white flocculent precipitate of hygromycin B separated. This preparation was about 80% pure. Final purification was accomplished by adsorption of the antibiotic on a cation-exchange resin (Amberlite IRC-50 in the Li^+ cycle) followed by elution with 2% ammonium hydroxide.

(1) R. C. Pittenger, R. N. Wolfe, M. M. Hoehn, Phoebe Nelms Marks, W. A. Dailey and J. M. McGuire, *Antibiotics and Chemotherapy*, **3**, 1268 (1953).

(2) R. L. Mann, R. M. Gale and F. R. Van Abeele, *ibid.*, **3**, 1279 (1953).

(3) Robert L. Mann and D. O. Woolf, THIS JOURNAL, **79**, 120 (1957).

(4) Max C. McCowen, Frank O. Gossett, Maurice E. Callender and Milo C. Brandt, to be published.