ingested large quantities of nicotinic acid did not obscure the test for porphyrins when it was added to urine with an increased quantity of porphyrin.

Twelve control subjects showed a transitory increased porphyrinuria, which, however, was not as great as that found in 7 painters before the administration of nicotinic acid.

The results summarized in Table I show that even in cases of long standing increased porphyrinuria such as that resulting from exposure to lead for periods of at least 15 years, nicotinic acid in the amounts given readily reduced the degree of porphyrinuria. This finding supports the observation of Spies, Gross and Sasaki[1] that the porphyrinuria present in pellagra and certain other diseases decreased rapidly following the administration of nicotinic acid. Since the porphyrinuria was reduced to its minimal quantities only after a number of days, rather than immediately after the nicotinic acid was administered, it seems that this substance does not mask the porphyrinuria but plays a rôle in the metabolism of porphyrin.

Summary and Conclusions. 1. The urine of 7 painters showed increased porphyrinuria as compared with the amount present in the urine of 45 control subjects. 2. In every instance this increased porphyrinuria decreased following the administration of nicotinic acid. 3. These studies suggest that nicotinic acid may play a rôle in the metabolism of porphyrins.

9824 P

A Complement-binding Antigen in Extracts of the Brown-Pearce Carcinoma of Rabbits.

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Previous studies[1] have shown that a complement-binding antigen is present in extracts of virus-induced rabbit papillomas, and that this antigen is closely associated with the virus causing the growths, if not identical with it.[2] In the present work it has been learned that extracts of a transplantable epithelioma of rabbits[3] also contain

1 Kidd, John G., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 612.

2 Kidd, John G., PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 657.

3 Brown, W. H., and Pearce, L., *J. Exp. Med.*, 1923, **37**, 601.

a complement-binding antigen which will react specifically with the sera of rabbits bearing the tumor, or in which it has retrogressed.

Procedures. The antigen-extracts were prepared from tumor tissue obtained from vigorous transplants in muscle or testicles, or from metastases in the omentum or diaphragm. The tissue was kept frozen at —20°C. or in 50% glycerol-Locke's solution at about 4°C. until needed, then ground with sand and suspended in isotonic saline (proportions usually 1:20), kept overnight in the refrigerator, and centrifugalized at 3500 r.p.m. in the angle-head centrifuge for 10 to 20 minutes. The supernatant fluids were then inactivated at 56°C. for 30 minutes and used in the tests.

Tests have been made using extracts of the Brown-Pearce tumors of 8 rabbits and sera from 34 animals bearing the growths or in which these had recently retrogressed. They were set up precisely as in the experiments with the papilloma virus, using 2 units of complement and sera diluted 1:4. Control tests for anticomplementary effect were made with double amounts of all materials.

Outcome of the Tests: Extracts of the vigorously-growing Brown-Pearce tumors of 7 rabbits bound complement notably well when mixed with sera of rabbits carrying the growths, but extracts of the necrotic tumor tissue of an 8th animal failed to do so.

Twenty of the 34 sera obtained from rabbits bled 28 days or longer after the tumor had been transplanted to them bound complement specifically when mixed with extracts of the growths, the most effective sera coming from rabbits which had borne 6 or 8 large tumors for some weeks.

None of 29 sera obtained from normal or pregnant rabbits, or from rabbits immune to vaccinia or Virus III, has bound complement specifically when mixed in the routine way with extracts containing the complement-binding antigen; nor has specific fixation occurred when active Brown-Pearce antisera were tested with extracts of rabbit embryos or of normal rabbit skin or testicles, or with extracts of rabbit testicles heavily infected with Virus III. In an experiment to test further the specificity of the reaction, the sera of 4 rabbits carrying the Brown-Pearce tumor bound complement when mixed with antigens derived from growths of this sort, but failed to do so when mixed with antigens derived from virus-induced rabbit papillomas; whereas 4 other sera, procured from rabbits immune to the papilloma virus, fixed complement in high titer when mixed with papilloma antigens, but did not do so at all when mixed with antigens derived from the Brown-Pearce tumors.

Properties of the Complement-Binding Antigen: Extracts of the Brown-Pearce tumor heated to 60°C. for 30 minutes retained their capacity to bind complement when mixed with active sera, but this was almost or entirely lost after heating them at temperatures of 66°C. or higher for the same period.

In many experiments the antigen has been found to pass through Berkefeld V filters, although some of it was retained by them as a rule. Most of it was held back by Berkefeld W candles, and practically all by single Seitz EK disks.

The complement-binding antigen has been deposited by centrifugation in two experiments. In the first, extracts of the Brown-Pearce tumors were put into a high-speed, angle-head International centrifuge, which was then run at about 18,000 r.p.m. for 6 hours; and in the second, similar extracts were spun in an air-driven ultracentrifuge at about 30,000 r.p.m. for 3 hours. The supernatant fluids in both cases were devoid of ability to bind complement when mixed with active antisera; whereas the sediments, resuspended in the original volumes of saline, had approximately the same capacity to fix complement as portions of the extracts which had not been centrifugalized.

To test the reaction of the complement-binding antigen to changes in pH, extracts of the omental metastases of 2 animals were prepared as usual, and portions of each were adjusted to the pH levels stated below with NaOH and HCl, some being saved untreated as well. All were incubated for 1 hour at 37°C., kept in the refrigerator overnight, inactivated at 56°C. for 30 minutes, and finally brought back to pH 6.8 to 7.0. When mixed immediately thereafter with active sera and tested for capacity to bind complement, those portions which had been subjected to pH 2.5 and 4.5 did not bind it at all; whereas the control portions, which had remained at pH 6.0 and 6.8 respectively, bound it completely. The materials subjected to pH 8.5 still bound complement, but not quite so well as did the controls; while those portions which had stood at pH 10.0 had little capacity to bind complement, and those kept at pH 11.5 had none whatever.

Tumor extracts containing the complement-binding antigen in large amount have failed to give rise to any lesions when inoculated intradermally or rubbed into the scarified skin of normal rabbits.

Summary and Comment: A substance is present in extracts of the Brown-Pearce tumor which binds complement specifically when mixed with the sera of rabbits bearing the growth, or in which it has retrogressed. The substance is readily distinguishable from the

complement-binding antigen which can be extracted from the virus-induced papillomas of rabbits, though it resembles the latter in its general traits. Further work is under way to determine the significance of the findings.

9825 P

Germicidal Efficiency of Synthetic Phenolic Compounds Tested by the Improved Tissue Culture Method.

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In the preceding paper of this series[1] an improved method was proposed for testing and rating germicides. The compounds which were rated by the method included: iodine, Hexylresorcinol, Metaphen, phenol, Mercurochrome, and Merthiolate.

The same method was followed for the evaluation of several synthetic organic compounds belonging to the phenolic group. The compounds were tested for their effect on the growth of living embryonic chick tissue as well as for their ability to kill bacteria. A number known as the toxicity-index was determined which is defined as the ratio of the highest dilution of disinfectant showing no growth of embryonic tissue in 10 minutes to the highest dilution required to kill the test-organism in the same period of time. The tests were run at a temperature of 37° C. in the presence of a standard amount of organic matter. Theoretically the smaller the index the more nearly perfect the germicide.

The following compounds were tested and compared: *o*-n-hexylphenol, *p*-hydroxydiphenyl sulfide, and *p*-hydroxyphenyl-n-amyl sulfide. Since phenol and Hexylresorcinol are also phenolic compounds they are included for comparison.

Effect of germicides on bacteria and tissue. The compounds were tested against both *Staphylococcus aureus* (gram +) and *Eberthella typhosa* (gram —). The killing concentrations of the germicides for tissue and bacteria and their toxicity-indices after 10 minutes' exposure at 37°C. are given in Table I.

The phenolic compounds that have been tested show relatively small toxicity-indices indicating that they are efficient germicidal

[1] Salle, A. J., McOmie, W. A., Shechmeister, I. L., and Foord, D. C., PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 694.