fresh mice, but the filtered pooled intestinal contents of the same stock animals, similarly inoculated, induced in 6 of 8 mice the characteristic encephalomyelitis. Intracerebral passage of the brain and filtered intestinal contents of the latter was again positive. It would now appear that (a) either normal mice harbor this virus in the intestinal contents, or (b) these contents of mice in general are non-viral but can activate, after intracerebral inoculation of filtrates, a latent carriage of virus, thereby inducing clinically apparent disease. If this occurs, one may postulate that the first test-mouse which reacted to inoculation of the filtered intestinal contents and thus initiated the present series of transmissions may have had its infection brought about in this way. Other substances introduced into the brain of mice do not, as a rule, cause this effect in view of the extremely low incidence of the malady observed among thousands of mice used in this laboratory for experimental transmission of various agents.[2]

10702

Observations on the Mode of Action of Sulfapyridine on Pneumococcus.*

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The introduction of successful chemotherapeutic measures against infection gives rise to speculations as to the mode of action involved. Whitby[1] reported on the effectiveness of sulfapyridine against certain types of the pneumococci and stated that "It appears to exert a definite action on the capsule of the pneumococcus." This view has not been accepted by those who have worked extensively with the drug, although the conception that the capsule is injured in some way, so that typing is either not possible or is rendered difficult after administration of sulfapyridine, has been retained by some clinicians.

Long[2] and his associates have expressed the view that the drug

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1 Whitby, L. E. H., *Lancet*, 1938, **1**, 1210.

2 Long, P. H., Bliss, E. A., and Feinstone, W. H., *Penn. Med. J.*, 1939, **42**, 483.

"brings changes in the morphology and somatic elements of the pneumococcus and, in certain instances, so injures these micro-organisms that they are susceptible to phagocytosis by the monocytes and clasmatocytes present in the peritoneal exudate."

In the concentration of the drug used in his experiments, Fleming[3] reported that sulfapyridine was bacteriostatic but not bactericidal and that leucocytes were necessary for the destruction of pneumococci and streptococci in blood containing sulfapyridine.

The following experiments were made in a further attempt to determine the manner in which sulfapyridine affects the pneumococcus or stimulates the body defenses against invasion by that micro-organism.

I. *Effect of Sulfapyridine on the Pneumococcal Capsule.* Mice weighing approximately 20 g were given 0.5 cc of a suspension containing 10 g of sulfapyridine in 100 cc of 10% gum acacia by mouth as described by Feinstone, Bliss, Ott and Long.[4] The first dose was given in the evening, and in the morning (about 8 hours later) a second dose was administered. This was followed immediately by an intraäbdominal injection of 0.5 cc of a 1-1000 dilution of a 14-hour broth culture of pneumococcus Type I. Peritoneal taps were made on each mouse at once after injection of the organisms, and each hour for 12 hours, and at 6-hour intervals thereafter until 36 hours had passed. During this time a satisfactory blood level of sulfapyridine was maintained in the mice by oral administration of the drug every 4 hours. This amount and method of administration was established as adequate by Long and Feinstone.[5]

Capsule-stains were made by the Hiss technic and the pneumococci present were typed by the Neufeld method from the material obtained by peritoneal tapping. In each case the capsule was found to be unaltered and the type of the organism, as determined by marked capsular swelling in the presence of homologous immune serum, was not changed.

Identical results were obtained when 0.5 cc of a 100 mg % suspension of the drug was injected into the animal's peritoneal cavity.

The experiment was repeated with Type III pneumococcus with similar results.

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[3] Fleming, A., *Lancet*, 1938, **2**, 564.

[4] Feinstone, W. H., Bliss, E. A., Ott, E., and Long, P. H., *Bull. Johns Hopkins Hosp.*, 1938, **62**, 565.

[5] Long, P. H., and Feinstone, W. H., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 486.

The same culture was inoculated into infusion-broth containing about 2% sterile ascitic fluid and 10 mg % sulfapyridine. As soon as the growth became visible, capsule-stains were made and the organisms showed a positive *"quellung"* reaction. Over a period of 24 hours no apparent change occurred in the capsules and no difficulty was encountered in typing by Neufeld's method.

II. *Effect of Sulfapyridine on Phagocytosis.* Sterile peritonitis was induced in a group of mice weighing 18-20 g each by injection of 2.0 cc of a suspension of 1% aleuronat and 1% gum tragacanth. Sulfapyridine was administered to these mice at 4- to 6-hour intervals, *per orum* as in the preceding experiment. Eight hours after injection of the foreign matter intraäbdominally and immediately following the second dose of sulfapyridine, the mice were given intraäbdominal injections of a 1-1000 dilution of a 12-hour broth culture of pneumococcus Type I.

Peritoneal taps were made at 2-hour intervals for 36 hours and the blood level of sulfapyridine was maintained by continued oral administration of the drug at the stated intervals.

Duplicate smears made from the peritoneal fluid were stained with Wright's and with Gram's stain. In these smears evidence of phagocytosis was looked for as well as careful examination of the cells for any visible somatic or morphological changes. A second group of mice was treated in the same manner except they were given no sulfapyridine and a third group was given a single intraabdominal injection of 1500 units of Type I pneumococcus antiserum. The fourth group of mice received an intraäbdominal injection of 0.5 cc of 100 mg % sulfapyridine and 1000 units of antiserum.

There was no significant difference found in the numbers of leucocytes acting as phagocytes nor the number of bacteria engulfed by the phagocytes in the smears taken from the mice receiving sulfapyridine and those "unprotected" control mice. The mice in the groups that received antiserum showed a high percentage of leucocytes acting as phagocytes and the phagocytes contained large numbers of bacteria.

It was noted that what phagocytosis took place in the exudates from the control and sulfapyridine-groups occurred almost exclusively in the monocytes while in the groups getting antiserum the polymorphonuclear leucocytes also acted as phagocytes.

An increase in the number of pneumococci in the peritoneal exudate was observed in the control group of mice beginning with the

first tap and increasing with each succeeding examination. All mice in this group were dead in less than 20 hours.

The number of bacteria in the exudate from the mice getting sulfapyridine increased slightly during the first 6 hours but decreased rapidly after that. It was with extreme difficulty that any pneumococci were found either intra- or extra-cellularly after 10 hours, none being found after 18 hours. These mice survived until 3 or 4 days after the final administration of the drug. At autopsy pneumococci were recovered in large numbers from the peritoneal exudate and from the heart-blood.

In the mice treated with immune serum there was rapid ingestion of the diplococci. All organisms found after the fourth hour were intracellular and these gradually disappeared. These mice survived.

To determine the effect of sulfapyridine on the phagocytosis of pneumococci *in vitro*, tests were made using mixtures as indicated in Table I. The leucocytes were taken from guinea pigs after inducing a sterile peritonitis in the usual manner. The culture of pneumococcus Type I was grown for 15 hours in infusion-broth containing ascitic fluid. The sulfapyridine was added to give a final concentration in the mixture of 10 mg %. The volumes were made equal by the addition of physiological saline. The mixtures were incubated at 37°C for 30 minutes and except in tubes 1 and 2, smears made at that time. In tubes 1 and 2 either leucocytes or bacteria were added, as indicated, and incubated for another 30 minutes before making the smears. The slides were stained by Wright's and Gram's technic.

The results of this experiment indicated that phagocytosis was not enhanced by the presence of sulfapyridine. No appreciable in-

TABLE I.

Phagocytic Mixtures	Avg No. of pneumococci in 100 leucocytes	% of leucocytes acting as phagocytes
Leucocyte suspension, Sulfapyridine 10 mg %, Salt soln. (incubated 30′ at 37°C.) Pneumococci added. Re-incubated	3.0	2.0
Pneumococci, Sulfapyridine 10 mg %, Salt soln. (Incubated 30′ at 37°C.) Leucocytes added. Re-incubated	3.2	4.5
Leucocyte suspension, Pneumococci, Sulfapyridine 10 mg %, Salt soln.	3.1	3.5
Leucocyte suspension, Pneumococci, Immune serum, 0.1 cc—300 units, Salt soln.	240	60
Leucocyte suspension, Pneumococci, Immune serum, Sulfapyridine, Salt soln.	230	62
Leucocyte suspension, Pneumococci, Salt soln.	2.9	1.2

crease in the number of leucocytes acting as phagocytes or the number of bacteria engulfed by the phagocytes was noted in those mixtures containing sulfapyridine alone and the control containing none of the drug. In smears made from mixtures containing immune serum considerable phagocytosis was found.

III. *Bacteriostatic and Bactericidal Effect of Sulfapyridine.* Infusion-broth with ascitic fluid containing 10 mg % of sulfapyridine and similar media without the drug were inoculated with approximately 50,000 organisms from a 12-hour broth culture of pneumococcus Type I, as determined by plating on infusion-agar containing 10% defibrinated rabbit blood. Di-hourly plates were made and smears prepared and stained by Gram's stain, Hiss capsule-stain and with dilute carbol fuchsin for several hours.

After 12-14 hours the cultures containing the sulfapyridine showed a decreasing number of cultivable pneumococci, but no dissociation was noted. The lag phase of both cultures was negligible; the logarithmic phases of the cultures progressed nearly in parallel up to 14 hours when it continued in the case of the control culture for several hours but ceased in the case of the sulfapyridine culture. The phase of decline was somewhat accelerated in the case of the culture containing the drug.

Gram's stains showed typical Gram-positive diplococci.

Capsules were found consistently in both cultures, and no morphological or somatic changes could be detected in the organisms stained with weak carbol fuchsin.

Conclusions. 1. Sulfapyridine did not affect the capsule of the pneumococcus either *in vivo* or *in vitro* in such a way as to be ascertainable by capsule-staining or by affecting the *"quellung"* reaction in the presence of immune serum. 2. *In vivo* and *in vitro* experiments using sulfapyridine in pneumococcal infections or pneumococcal cultures containing leucocytes did not indicate an increase in phagocytosis of the diplococci in the presence of the drug. 3. *In vitro* there was only slight bacteriostatic effect of 10 mg % sulfapyridine in broth cultures of pneumococcus Type I until the culture neared the close of the logarithmic phase of growth. At that time a bactericidal effect was apparent along with increased bacteriostasis and the length of the phase of decline was decreased markedly. 4. *In vivo* a similar effect was noted. In mice treated with sulfapyridine there was a short period of increase in the number of organisms in the peritoneal exudate followed by a rapid decrease in the number of bacteria found until, after 18 hours, only a few organisms remained. In several days, after stopping administration

of the drug, the treated mice died and pneumococci were recovered from the heart-blood and peritoneal exudate.

5. Complete bactericidal action was not demonstrated.

6. It would seem that sulfapyridine may owe its beneficial effect in pneumococcal infections to its bacteriostatic action which may permit antibodies to be formed by the body or to be acquired passively, or allow the inhibited organisms to be disposed of by phagocytosis at the normal rate.

10703 P

Conversion of Methionine to Cystine: Experiments with Radioactive Sulfur (S^{35}).*

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A considerable amount of evidence pointing to the idea that methionine, when fed, may be converted into cystine in the animal organism has been brought forth.[1] Further evidence in support of this concept is afforded by the present experiments in which it is shown that cystine containing the radioactive sulfur isotope (S^{35}) was isolated from rats that were fed methionine containing S^{35}.

The methionine was synthesized from sulfur that contained S^{35} by a modification of the procedure described by Patterson and du Vigneaud.[2] † It was administered to young rats that were maintained on a low cystine diet. Animals 20 and 24 received 0.15% of the S^{35}-containing methionine daily in their diet, while rat 22 was given daily 15 mg of the same methionine subcutaneously. Rats 32, 34, and 37, maintained on the same basic diet, were fed 0.15% of ordinary methionine daily plus 0.10% of sodium sulfate that contained

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1 Womack, M., Kammerer, K. S., and Rose, W. C., *J. Biol. Chem.*, 1937, **121**, 403; Brand, E., Cahill, G. F., and Harris, M. M., *J. Biol. Chem.*, 1935, **109**, 69; Dawbarn, M. C., *Austr. J. Exp. Biol. Med. Sci.*, 1938, **16**, 159; Beach, E. F., and White, A., *J. Biol. Chem.*, 1939, **127**, 87.

2 Patterson, W. I., and du Vigneaud, V., *J. Biol. Chem.*, 1935, **111**, 393.

† The radioactive sulfur was kindly furnished by the Radiation Laboratory of the University.