which is the presence of inhibitor substances.

Summary. Attempts were made to experimentally demonstrate a circulating factor in the blood of normal people and of patients with myasthenia gravis before and after exercise to a fatigued state, which would depress the response of prostigminized frog and leech muscle to acetylcholine. Before the addition of acetylcholine to the perfusion fluid it was found that neither normal serum nor serum from myasthenic donors had any effect upon the prostigminized leech or frog muscle. Serum, whether from a normal or myasthenic donor, had no significant effect upon the acetylcholine sensitivity of either frog or leech muscle. Although hemolyzed blood from both normal and myasthenic donors increased the acetylcholine sensitivity of prostigminized leech and frog muscle, there was no quantitative difference in the effect of the two types of hemolyzed blood. A circulating factor depressing the acetylcholine sensitivity of frog and leech muscle could not be demonstrated with our methods in myasthenic patients. Some reasons for the non-detection of this hypothetical factor and some theories as to the etiology of myasthenia gravis were discussed.

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Penicillin: Miscellaneous Pharmacological Results.*

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Penicillin has surprised all investigators in its non-toxicity. Various phases of acute and chronic toxicity, and *in vitro* activity, are reported in this paper. The results corroborate those of van Dyke[1] in the paucity of adverse pharmacodynamic effects.

1. *Effect on Excised Organs.* The contractions of strips of pregnant guinea pig uterus in an excised-organ bath were not affected by 1, 2 or 10 cc of a solution containing 1500 u of penicillin per cc. Similarly, several strips of rabbit abdominal aorta were not influenced by from 1 to 5 cc of the same penicillin, while 1 cc of a 1:5 million dilution of epinephrine produced contraction.

2. *Vomiting in Pigeons.* Two pigeons were given penicillin intravenously, 4000 and 7500 u respectively in 5 cc of water, without the production of vomiting, or other symptoms, in 30 minutes.

3. *Anticatalytic Effect.* Two hundred and fifty individual tests were made of the *in vitro* effect of penicillin on the liberation of oxygen from hydrogen peroxide by a catalyst.[2] The results were similar, irrespective of whether Fuller's earth or collargol served as the catalytic agent. Penicillin from 6 different sources, in high concentrations, invariably inhibited the liberation of oxygen, while in low concentrations (50 units or less in the system), it produced no effect. It was at first hoped that

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[1] van Dyke, H. B., PROC. SOC. EXP. BIOL. MED., 1944, **56**, 212.

[2] Hanzlik, P. J., and Cutting, W. C., *Science*, 1943, **98**, 389.

this might be a means for assaying penicillin, but inactivation of the penicillin by incubation at 37° C for 4 days, by boiling, by autoclaving at 15 lb pressure for 20 minutes, and by autoclaving penicillin acidified to pH 2, did not greatly affect the inhibitory power on the production of oxygen. This suggests that the inhibitory substance is not penicillin, although it could conceivably parallel the original penicillin content. A sample experiment is shown in Table I, in which oxygen was available in each tube from 1 cc of 1% hydrogen peroxide plus 1 cc of 0.02% collargol.

TABLE I.
The Inhibition of Catalytic Action by Penicillin and Inactivated Penicillin.

Test agent	Height of gas column produced	Height of gas column produced after autoclaving penicillin
Control	6.0 cm.	....
"	5.6	....
Penicillin, 5000 u.	0	0 cm.
" 500 u.	0.8	0.3
" "	0.7	0.4
" 50 u.	5.6	5.2
" "	5.3	4.8
" 5 u.	4.4	5.2
" "	5.0	5.4
" 0.5 u.	4.8	5.7
" "	4.8	5.9

Ten per cent solutions, or saturated solutions, of several agents which were considered to be possible inhibitors of penicillin were added to the system in 0.5 cc quantities, after neutralization. Whereas 6.2 cm of gas were produced in the control tube, all the tubes containing penicillin (500 u) showed complete, or almost complete, inhibition, the greatest quantity of gas produced being only 0.4 cm (superoxol). The agents included sodium benzoate, potassium permanganate, para-aminobenzoic acid, sodium citrate, Solution of Formaldehyde, U.S.P., hydrogen peroxide 30%, sodium salicylate, urea, propylene glycol, acetone, and ferric chloride (unsatisfactory).

Tyrothricin was found not to affect the production of gas in the catalytic system.

4. *Acute Meningeal Irritation.* Because of clinical suggestion that penicillin might be irritating to the meninges, it was injected intracisternally into 2 dogs, one being given 2000 u in 2 cc of solution, and one 200 u in 2 cc of solution 5 times during 6 days. Estimations of the spinal fluid protein were made on the first, second and fifth days, with the following results: Dog 1, receiving 2000 u daily, 62, 41 and 19 mg per 100 cc; Dog 2, receiving 200 u daily, 85, 88 and 187 (bloody) mg per 100 cc; Control Dog 3, given 1 cc physiological salt solution daily, 62, 147 (bloody), 99 and 209 (bloody) mg per 100 cc. From this it was concluded that the greater concentration of penicillin did not produce an increase in spinal fluid protein.

On the 7th day, the dogs were sacrificed. The brain of the dog which had received 200 units of penicillin daily showed slight hemorrhages at the site of the injections and several collections of mononuclear cells in the meninges over the base of the brain. These latter may have been in response to the bleeding. The brain of the dog which had received 2000 units of penicillin daily showed only a very few collections of mononuclear cells, similar to those in the first dog, and was otherwise not abnormal, from which it was concluded that the stronger penicillin solution did not damage the meninges.

5. *Chronic Toxicity.* One rooster was given 500 to 1000 u of penicillin intramuscularly daily except Sundays for 22 days. At autopsy no gross abnormalities were seen. Four rats were each given 500 u of penicillin subcutaneously in 5 cc of physiological salt solution daily except Sundays for 6 months. During this time they appeared healthy and raised several litters. Examination of conjunctival blood vessels under magnification at 2 months showed no abnormalities of blood flow. When sacrificed at the end of the period of injection, pathological sections were made of all the principal organs and tissues. Locally at the site of injection there were numerous old and new hemorrhagic areas, excessive accumulations of fibrous tissue and some foreign body giant cells. The injections were not made with sterile precautions, and the above changes may have been due to trauma and the accidental introduction of microorganisms and foreign particles. Otherwise, the only abnormal pathological changes were a few clumps of lymphocytes in the brain of one of the animals

and an excessive number of eosinophils in the bronchial walls, a finding not uncommon in control rats. The conclusion is that chronic toxicity of penicillin in rats is not demonstrable with the doses used.

6. *Percutaneous Absorption.* Penicillin was applied to the freshly clipped abdominal skin of rabbits by gentle rubbing on two occasions. For the first trial, 1000 units of penicillin were dissolved in 5 cc of monoethylether of diethylene glycol (carbitol) and applied over 15 minutes; for the second, 5000 units were dissolved in 2.5 cc of the same vehicle and applied over 1 hour. In neither case was there any measurable penicillin in the blood at the end of the period of application. A mixture of 4000 units of penicillin in 0.05 gm of zephiran was administered sublingually to a man. No penicillin appeared in the urine during the next 45 minutes.

Summary. In spite of the lethal effect which penicillin exerts on bacteria, power to alter or poison normal physiological processes significantly has not been demonstrated. It has no demonstrable effects on contraction of the isolated aorta or pregnant uterus, or on the vomiting reflex of pigeons; it produces no serious meningeal irritation; and, in small doses, it produces no chronic tissue changes in rats. Although the penicillin used inhibited the liberation of oxygen from hydrogen peroxide by catalysis, the inhibition persisted after inactivation of the penicillin and, therefore, may be due to some other substance in the impure products. Monoethylether of diethylene glycol did not promote its percutaneous absorption.

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Collodion Particle Adsorption of Equine Encephalomyelitis Virus.

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The flocculation of talcum powder or of organisms such as *Proteus vulgaris* or *Eberthella typhi* in *Escherichia coli* filtrate upon the addition or anti-coli serum was reported as early as 1898 by Nicolle.[1] That protein is adsorbed upon various particles has been shown by changes in physical properties such as isoelectric point or cataphoretic mobility.[2-4] The agglutination of collodion particles as a result of the reaction of the adsorbed protein with specific antiserum was observed by Jones[5] and by Freund.[6]

Collodion particle suspensions have been used in many studies involving their adsorption of antigen.[7, 8] In most cases, protein solutions containing relatively large amounts of antigen have been employed for adsorption upon the collodion particles. The adsorption of virus antigens from tissue suspensions, where the specific antigen is in very small amount, upon cells of *Serratia marcescens* with the demonstration of agglutination by virus antisera has been reported by Roberts and Jones[9] and Weil.[10] Later, Pearson[11] was unable to show agglutination with specific antisera and collodion particles or cells of *S. marcescens* which had adsorbed material from

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