ischemia nor ischemic pain involving the biceps has any influence on the triceps reflex. It is concluded that muscle pain interferes with muscular coordination through influences on spinal and possibly cortical centers.

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Pharmacological Observations on Crystalline Sodium Penicillin.

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Through the kindness of Dr. O. Wintersteiner, of the Division of Organic Chemistry of this Institute, a small quantity of twice-crystallized Na Penicillin-G was made available for pharmacological experiments which had to be restricted owing to the great scarcity of this pure antibiotic. Its potency was 1650 Oxford units per mg.

Results. Hemolysis of washed rabbit erythrocytes suspended in 0.15 M NaCl or in 0.15 M Na penicillin was compared at 37° after the addition of one part of erythrocyte-suspension in isotonic saline equivalent to original blood to 10 parts of a solution of NaCl or of Na penicillin. No hemolysis occurred during 3 hours. After 17 hours, the erythrocytes suspended in Na penicillin solution had become converted into a chocolate-colored granular mass not easily homogenized; the suspending fluid was water-clear. Microscopically the brown material appeared to consist of granules about 0.5 micron in diameter; a few irregular "ghosts" could be found. No further change occurred during a total period of 48 hours after which only 4% of the original antibiotic activity remained in the supernatant fluid.

Dr. R. J. Dubos suggested that possibly adsorption of penicillin on the rabbit erythrocytes had occurred. In another experiment, the suspending fluid contained 1 mg (1650 units) of Na penicillin per ml together with sufficient NaCl to make the solution equivalent to 0.15 M NaCl. Erythrocyte suspensions with and without penicillin as well as Na penicillin-NaCl solutions without erythrocytes were incubated at 37° for 2 hours in duplicate. There were suitable control tubes from which erythrocyte suspension in saline or the supernatant fluid of such a suspension was added to incubated Na penicillin solution at the time of the antibiotic tests. No hemolysis occurred. Within the limits of error (± 20%) of the determination of antibiotic activity, which was kindly estimated by Miss C. M. McKee, there was no loss of activity in any tube including those from which the erythrocytes had been removed by centrifugation at the end of the period of incubation. It appears unlikely that important amounts of penicillin are adsorbed by rabbit erythrocytes suspended in isotonic saline solution.

Dr. Eric G. Ball, of Harvard Medical School, performed a careful experiment to determine whether yeast cells, duck erythrocytes, or rat liver slices consume oxygen at an altered rate if 3400 units (2.05 mg) of Na penicillin replace an osmotically equivalent amount of NaCl in each ml of Locke's solution containing phosphate buffer at pH 7.33 and glucose as the substrate. All observations were made at 37° C. The solution of Na penicillin was added to the suspensions after a control period of 20 minutes. The oxygen-consumption of other control suspensions was followed throughout the experiment. The total period of observation was 214 minutes. The Na penicillin caused no change in any of the 3 tissues with regard to the rate of oxygen-consumption.

The results of representative tests with a few isolated organs are illustrated in Fig. 1-3. A detailed description of the individual trac-

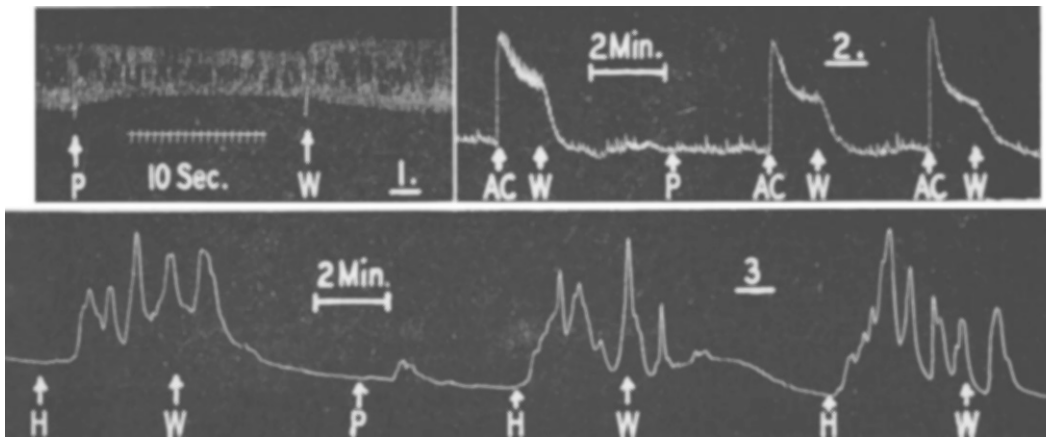

FIG. 1. Effect of Na penicillin on the isolated frog heart perfused by Straub's method. Control perfusing fluid contained 0.001 M $CaCl_2$, 0.002 M KCl, 0.003 M $NaHCO_3$, and 0.111 M NaCl. At P the control fluid was replaced by one containing 0.111 M Na penicillin but otherwise identical. At W the control fluid replaced the solution containing penicillin. The only noteworthy change was a decrease in the amplitude of contraction.

FIG. 2. Isolated guinea-pig ileum. Bath fluid: solution of van Dyke and Hastings containing 0.75 mM $CaCl_2$. At AC, acetylcholine Cl was added to final concentration of 1 to 6.67×10^8; at W the preparation was washed without exposure to air; at P, Na penicillin was added to a final concentration of 1 to 5.46×10^3. Note that the Na penicillin neither caused any change in spontaneous movements nor interfered with the response to acetylcholine Cl.

FIG. 3. Isolated virgin guinea-pig uterus. Same bath fluid as in Fig. 2. At H, an extract of U.S.P. standard posterior-lobe pituitary powder was added in a concentration equivalent to 1 part of powder to 10^8 parts of bath fluid. At P, Na penicillin was added to a final concentration of 1 to 5.46×10^3. W indicates washing without exposure to air. A trivial uterine contraction followed the addition of Na penicillin which did not alter the uterine response to posterior pituitary extract.

ings is given under the figures. These tests confirm the generally-held view that penicillin has a remarkably low toxicity.

The limited quantity of Na penicillin available permitted only a few toxicological observations in mice. Male mice which weighed 21.6-24.6 g initially were used. Na penicillin was injected intravenously as an isotonic solution in comparison with control injections of isotonic saline (both 0.15 M.) over a continuous period of about 20 minutes without anesthesia. Single doses of 1,690,000-1,770,000 units per kg (2 mice) were without apparent effect on behavior, body weight, hemoglobin, total white and red cell counts. Serial electrocardiographic tracings were made before, during and after intravenous injection of 3,820,000 units per kg during a period of 21 minutes. Barbital solution (pH 8.4) was used to produce anesthesia (15 ml of 0.02M solution per kg intraperitoneally); 6 hours later 0.6 mg of picrotoxin per kg was administered subcutaneously to facilitate recovery. A control mouse was similarly treated except that a similar volume of isotonic NaCl instead of isotonic Na penicillin was injected intravenously. (Another control mouse was killed after tracings had been made.) The heart rates changed as follows:

Solution injected	Heart beats per minute		
	Control	at end	30 minutes later
Na penicillin	280	260	360
NaCl	180-260	200-220	160-200

There were no obvious differences in the electrocardiographic patterns which were recorded from needle electrodes inserted subcutaneously in the fore-legs. Both mice recovered fully and have continued to gain weight.

A fourth mouse received a total dose of 9,790,000 units per kg during 7 days (1,320,000-1,870,000 units per kg were injected

once daily intravenously every day but the second). Its weight dropped from 22.8 g to 19.1 g but returned to the pre-injection level 16 days after the last injection. It has since gained satisfactorily. The only other grossly apparent untoward sign was necrosis of one side of the tail probably owing to thrombosis at a site of injection. Control mice receiving saline intravenously recovered their pre-injection weights 6-10 days after the last injection and had no necrosis of the tail. Hematological changes were detected only in the mouse receiving the 6 repeated injections of Na penicillin. The total red-cell count fell to 4,300,000 (counts in control mice were 9,900,000 to 12,600,000) and was still below normal (6,800,000) 16 days after the last injection. A week later the number of erythrocytes per cmm was 9,700,000 and was regarded as normal. The leucocyte count varied considerably (12,000-27,000 per cmm); however, simiiar variations were encountered in control mice. On 2 out of 4 occasions (12 and 23 days after the last injection) there appeared to be an abnormal increase in the proportion of neutrophils (42-56%) at the expense of the lymphocytes which were reduced to 52-36%; the differential counts preceding each of these counts were less abnormal (26% neutrophils, 71% lymphocytes). There were 6-18% neutrophils and 81-93% lymphocytes in the blood smears of control mice.

Summary. Crystalline Na penicillin-G is not a hemolytic agent and is not adsorbed by rabbit erythrocytes suspended in isotonic saline solution. It does not alter the oxygen-consumption of yeast-cells, duck erythrocytes or slices of rat liver suspended in a solution containing 2.05 mg (3400 Oxford units) per ml. A high concentration (0.111M) causes a moderate reduction of the amplitude of each beat of the isolated frog's heart without affecting the rate; the effect is completely reversible. A concentration of 1-5500 in the bath fluid has no significant effect on the isolated ileum or uterus of the guinea pig and does not alter the response of the ileum to acetylcholine or of the uterus to posterior pituitary extract. A single intravenous dose as high as 3,800,000 units (2.32 g) per kilo is tolerated by the mouse as well as a corresponding volume of isotonic saline. One mouse received 6 intravenous injections in 7 days (total dose: 9,800,000 units or 5.93 g per kilo). During the first 2 weeks following injection there was a transient loss of weight and a transient anemia.

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Demonstration of the Properties A, B, M, N and Rh in Red-Cell Stromata.*

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The discovery of the Rh factor in human blood[1,2] and its role in erythroblastosis[3,4] suggests the possibility of preventing the disease by specific desensitization. As a starting material for the preparation of Rh hapten, large quantities of packed red cells are available from blood and plasma banks which have been established in most of the large hospitals.

The intact erythrocytes contain only relatively minute quantities of the group-specific substances, A, B, M, N, and Rh, so that the therapeutic injection of whole blood would undoubtedly be ineffectual unless large quantities were injected. Such a procedure would

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1 Landsteiner, K., and Wiener, A. S., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 343.

2 Landsteiner, K., and Wiener, A. S., *J. Exp. Med.*, 1941, **74**, 309.

3 Levine, P., Katzin, E. M., and Burnham, L., *J. A. M. A.*, 1941, **116**, 825.

4 Levine, P., Burnham, L., Katzin, E. M. and Vogel, P., *Am. J. Obst. and Gyn.*, 1941, **42**, 925.