a certain elementary uncertainty.

Summary. With the administration of dicumarol to dogs there are no significant changes in the activity of antithrombin-II, and -III. Antithrombin-IV activity remained but no accurate quantitative measurements were made. In human subjects antithrombin-IV activity also remains and is probably not decreased. The thrombin-titer method of analysis shows that there are very marked changes in the activation rate of prothrombin soon after dicumarol is administered to human subjects. The addition of purified prothrombin does not restore the activation rate to normal. In contrast to human subjects the activation rate of prothrombin is not reduced in dog plasma when dicumarol is administered.

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Effects of 3-(o-Toloxy)-Propane-1,2-Diol (Mephenesin) and 3-(2-Methyl-6-Chlorophenoxy)-Propane-1,2-Diol (P105) on α-Excitability of Muscle (20932)

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3-(o-toloxy)-propane-1,2-diol (Mephenesin) has gained considerable attention as a muscle relaxant in both investigative and therapeutic fields within the last 10 years; its pharmacology has been reviewed by Berger(1). Lambooy(2), seeking more potent compounds for clinical use, synthesized 3-(2-methyl-6-chlorophenoxy)-propane-1,2-diol (P105) and found that although it was more toxic and less soluble than Mephenesin, it was twice as potent a paralyzing agent.

The experiments of Smith(3) and of others (1) on mono- and multi-synaptic reflexes indicated that Mephenesin specifically depressed internuncial neurones, but reports of conduction. rheobase, and chronaxie changes in peripheral nerve have been conflicting. Berger(4) failed to find any skeletal muscle changes produced by Mephenesin even in very high concentrations. Stephen and Chandy (5), however, found cat tibialis anticus action potentials to be reduced at dosages of 50 mg/kg. Mephenesin has also been found to cause contracture of frog rectus abdominis, as well as inhibition of acetylcholine-produced contracture of this muscle(6). In view of these various findings, it appeared of interest to study the effects of Mephenesin and of its analog, P105, on the electrical excitability of muscle.

Method. Blair(7) showed that the strength-duration curve of frog's muscle is adequately represented by the equation:

$$\log \frac{V}{V-R} = kt + \log \frac{K + k\alpha}{K} \qquad (a)$$

in which V is the strength of the stimulus, t is its duration, R is the rheobase, k is the coefficient of subsidence of the excitatory state, and in which the second constant $\frac{K + k\alpha}{K}$ represents physical rather than physiological factors. It can be shown, from the derivation of equation (a), that:

$$\frac{h}{K} = \frac{R}{k} \qquad (b)$$

when h is the particular value of the excita-

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tory state needed for excitation to occur, and K is the rate of build-up of the excitatory state per unit strength of stimulus. In these terms $\frac{h}{K}$ is a measure of membrane stability, increasing whenever the threshold value of h becomes large or whenever K, the efficiency of the stimulus, is decreased. Experimentally the values of R and of k can be readily determined, so that membrane stability as well as excitability can be followed under a variety of circumstances.

Sartorius muscles of *Rana pipiens* were removed and placed in Ringers solution at 5°C for periods of 4 to 24 hours. The Ringers solution was of pH 7.3-7.4, and contained per liter: 111.00 mM NaCl, 1.34 mM KCl, 1.36 mM $CaCl_2$, 1.33 mM NaH_2PO_4, and 5.35 mM Na_2HPO_4. Before the beginning of any experiment, a minimum time of 30 minutes was allowed for the muscle to attain room temperature. The muscle was stimulated through silver-silver chloride electrodes by means of a Grass stimulator, and threshold voltages were recorded for square waves of several different durations in the range of 0.02 to 50.0 milliseconds. Sometimes rheobase was considerably raised, and a relatively long pulse, around 500 milliseconds, was used in these cases to determine rheobase values. Threshold voltage for pulses of all durations was recorded at that value which caused the smallest visible muscular contraction. Since only muscle, or a, excitability was being studied, it was desirable to eliminate all possibility of motor nerve stimulation. Dihydro-β-erythroidine, an effective neuromuscular blocking agent(8), in a final concentration of 1:100,000, was added to all solutions to which the muscle was exposed during the course of any experiment. Three concentrations of Mephenesin and one concentration of P105 were used in a series of 22 experiments. The values determined in a given experiment were plotted as $\log \frac{V}{V-R}$ against t to yield straight lines in accordance with equation (a), the slopes of which were equal to values of k. Before a muscle was exposed to a drug solution, excitation measurements were made in Ringers until 3 successive determinations yielded the same results. These values of k and R in each instance were then considered standard for subsequent determinations of difference.

Results. Mephenesin in concentrations of 0.025, 0.05, and 0.1%, and 0.1% P105 consistently caused increases in R and k. These results are shown in Table I. Since definite changes were produced by 0.025% Mephenesin (or 1.35×10^{-5} M/1), it is probable that the minimal effective level was somewhat lower.

These drugs also caused an increase in the value of $\frac{R}{k}$, or muscle stability. Increases in $\frac{R}{k}$ always reflected changes in both components of this ratio. In almost every case R increased steadily on exposure to Mephenesin, but a few instances occurred in which an initial rise was followed after an hour or more by a small decrease. k, on the other hand, consistently increased and then decreased toward its initial value; the maximum was attained usually after 10 to 20 minutes, but sometimes after a period of one or more hours. Because of variability in the time course of these drug effects, the maximum increases produced in each muscle, regardless of exposure length, were found to express the results most satisfactorily.

In approximately half of the experiments, muscles were returned to Ringers after exposure to drugs, and recovery measurements were made. In no case was there complete return of both R and k to their original values within 2 hours, although the tendency was always in that direction.

In addition to the usual biological variations among frogs and among individual muscles, the results of these experiments are complicated by the problem of drug diffusion; the size and shape of each muscle are important variables. Were the tissue weight and dimensions known in each of the experiments, better evaluations could theoretically be made of the effects of a given drug concentration(9). Repetition of the above experiments on single muscle fibers would minimize diffusion difficulties and should certainly

TABLE I. Effects of Mephenesin and P105 on Excitability of Frog Muscle.

	No drug	Mephenesin .025%	Mephenesin .050%	Mephenesin .100%	P105, .100%
Max. change of R	−.01 (5)	.25 (3)	.39 (6)	.30 (4)	.27 (4)
Stand. error of mean	±.044	±.057	±.040	±.049	±.049
Probability	.83	<.001	<.001	<.001	<.001
Max. change of k	−.0067	.0657	.1157	.0716	.1583
Stand. error of mean	±.0043	±.0310	±.0219	±.0310	±.0268
Probability	.194	.055	<.001	.005	<.001

Each value for max. change of R or k obtained by averaging individual values from several experiments. No. of experiments in each case in parentheses. Avg control R value was .26 (22 exp.), stand. error of the mean, ±.021; avg control k value, .1120 (22 exp.), stand. error of mean, ±.0112.

yield more uniform results.

Discussion. The increases in stability $\frac{R}{k}$, which may just as well be called decreases in sensitivity to electrical stimulation, reflect corresponding increases in the ratio $\frac{h}{k}$, according to equation (b). Since it is not possible to determine h and K separately, one can only speculate about the meaning of changes in the ratio. Since k, the time constant of decay of the excitatory process, increased in the experiments above, one might believe that the time constant of build-up of the excitatory process, or K, also increased so that the whole process of excitation was hastened. If K grew larger, as the ratio $\frac{h}{K}$ increased, h must have undergone even larger increases. However, there is no information to indicate that k and K changed in the same direction, and one could just as reasonably propose that as k increased, K decreased; in this case h could remain constant.

In certain ways Mephenesin has behaved like potassium. Concentrations of Mephenesin of 5.4 x 10^{-3} M/l and higher were found to depolarize frog muscle(6), and it is reported that similar concentrations depolarized frog nerve(10). Changes brought about by this drug in the electrical properties of muscle also resemble those produced by potassium. Carleton(11) found that moderate amounts of potassium (30 and 40 mg %) added to Ringers, caused small and transient decreases in k and R, followed promptly by large increases in both quantities and a subsequent decrease in k. Except for the early decreases in k and R, this sequence of events is identical with that produced by Mephenesin.

One could propose that Mephenesin releases potassium from an organic complex in a manner similar to that proposed by Reginster(12) for acetylcholine. The released potassium could then be effective in depolarizing muscle and in changing its electrical properties. There are additional data which are in accordance with the released-potassium hypothesis: 1) Mephenesin, like acetylcholine, can cause contractures of rectus abdominis muscle, and 2) it can inhibit acetylcholine-produced contractures(6).

Summary. Mephenesin and P105 both consistently increase rheobase (R), the time constant of excitation (k), and stability $\left(\frac{R}{k}\right)$ of frog sartorius muscle.

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Erythrocyte-Modifying Capacity of *Shigella dysenteriae* (SHIGA) Antigen and Its Polysaccharide Component.* (20933)

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Studies carried out since 1947 have established the fact that antigenic components or products of numerous bacteria as well as of certain rickettsiae, fungi, and protozoa can be adsorbed by red blood cells, thus rendering these modified erythrocytes specifically agglutinable by homologous microbial antibodies. Some of these antigens are polysaccharides, others are proteins. The present study was undertaken to determine the erythrocyte-modifying capacity of *Shigella* (*S.*) *dysenteriae* (Shiga) antigen. Such an investigation appeared to be of particular promise, since this antigen has been studied in great detail(1-7) and both the whole antigen complex (a polysaccharide-phospholipin-protein (or protein-like) complex) and its polysaccharide component were available. It is the polysaccharide that gives the whole complex and, therefore, this microorganism its main serological specificity.

Material and methods. The whole somatic *Shigella dysenteriae* (Shiga) antigen as well as its polysaccharide fraction were prepared recently by Dr. W. T. J. Morgan of The Lister Institute of Preventive Medicine, London, England, according to methods described in 1937(3). Solutions containing 50 mg of the antigens per 100 ml of phosphate buffer† (8) were prepared. The following diagnostic antisera were used: 1) two *S. dysenteriae* (Shiga) (rabbit) antisera;‡ 2) one polyvalent *S. dysenteriae* and *S. paradysenteriae* (horse) antiserum.‡ In addition, 2 *S. sonnei* (rabbit) antisera,‡ 2 group-specific *Escherichia* (*E.*) *coli* 026 and 0111 (rabbit) and pneumococcus types 4 and 32 (horse) antisera§ were employed for control purposes. In order to remove erythrocyte antibodies, all sera were first absorbed with the respective untreated red blood cells which were used in the *S. dysenteriae* hemagglutination and hemolysis tests. Red blood cells were modified as described previously(9). The *S. dysenteriae* (Shiga) antigen (2 ml) in appropriate dilution was added to the sediment of thrice washed red blood cells; the cells were mixed, incubated for 30 minutes at 37°C, washed 3 times in physiological saline solution, and resuspended in 2 ml of the same diluent. The following procedure was employed for the demonstration of erythrocyte-modification. The antisera (0.2 ml) in serial dilutions were mixed with an equal volume of the modified red blood cells. The mixtures were incubated for 30 minutes at 37°C and the resulting hemagglutination was read after centrifugation at 1500 R.P.M. for 2 minutes. In the hemolysis experiments guinea pig complement‖ (0.1 ml of a 1:20 dilution) was added to the mixtures of antiserum and modified red blood cells. The tubes were incubated at

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