TABLE I.
Summary of RBC Counts of Central and Peripheral Blood in Various Laboratory Animals.

Animal	Age	No. of counts ♂	No. of counts ♀	Avg RBC count of central blood, ×1000	Avg RBC count of peripheral blood, ×1000	Probability
Rat	1-99 Days	64	101	5,297	5,729	.1–.05
Mouse	Adult	15	15	9,648	10,571	.1–.05
Guinea pig	"	12	8	6,422	6,160	.4–.5
Hamster	"	5	8	8,283	9,388	.1–.05
Rabbit	"	11	9	6,265	6,396	.6–.5
Dog	1 day	3	7	4,864	5,166	.6–.5
	Adult	6	14	6,284	6,529	.5–.4

heart, with about 15 minutes elapsing between samples. The order of sampling was reversed in about half the dogs in order to minimize the effect of splenic engorgement.

Peripheral and central red cell counts were also made in 10 newborn dogs at 1 day of age. No significant difference between central and peripheral red cell counts was found in young or adult dogs.

Discussion. In the study of central and peripheral red cell counts in 6 species, only the rat, mouse and hamster showed a difference between the two samples that approached significance. The statistical method used indicated, however, that the differences found in the averages could have been due to chance, since the probability factor was between 0.1 and 0.05, whereas a factor of 0.05 to 0.01 is necessary to demonstrate a high significance.

Summary. Red cell counts of blood secured from peripheral vessels were compared to counts from the heart or large vessels in the rat, mouse, hamster, rabbit, guinea pig, and dog. In none of these was a statistically significant difference found.

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Attempt at Percutaneous Introduction of d-Tubocurarine with a Direct Current into Muscles of Rabbits.*

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Curare affords a possible indicator to gauge the depth of penetration of positive ions through the skin under the action of a galvanic current (electrophoresis, iontophoresis), since 1) its physiological action is solely on muscle and can easily be detected, 2) it is active in small amounts, 3) it is dissociated, at least in acidic solutions, into positive ions suitable for transfer at the positive pole. Previous work on the depth of penetration using dyes (Harpuder,[1] Harpuder and Rein[2]) have shown visible dye only in the epidermis in humans, and in dogs and rabbits only a faint coloration of the superficial muscle layers under extreme conditions. The possibility remains, however, that in deeper layers the dye was decolorized or otherwise made undetectable as rapidly as it entered them.

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[2] Rein, H., *Z. f. Biol.*, 1926, **84**, 41.

TABLE I.
Threshold Values* of Single and Tetanizing Shocks for Peroneal-Dorsi-Flexor System Before and Following Electrophoresis with *d*-Tubocurarine.

Control leg				Test leg				pH of solution	Remarks
Single stimulus		Tetanic stimuli		Single stimulus		Tetanic stimuli			
a	*p*	*a*	*p*	*a*	*p*	*a*	*p*		
15	15	11	12	12½	12½	10	8½	4	Control: wet dressings
12½	12½	8½	8	15	15	12	12½	4	with tubocurarine
20	17½	15	15	12½	12½	12½	12½	4	Control: tubocurarine-
15	12½	10	10	8	7	6	7	4	free solution and elec-
20	20	17½	16	17½	17½	15	15	7	trophoresis
17½	20	15	16	22½	20	17½	15	7	
Avg $(p-a)$ +0.4		Avg $(p-a)$ 0		Avg $(p-a)$ −0.6		Avg $(p-a)$ −0.4			

* Threshold units are arbitrary, determined by ohmic resistance, and are proportional to intensity of stimulus.
a = before electrophoresis.
p = following electrophoresis.

Therefore, the present work using curare as a physiological rather than a histological indicator in the muscles is in order.

Nerve muscle preparations *in situ* were employed in rabbits anesthetized with sodium pentobarbital (30 mg/kg). The sciatic nerves were exposed bilaterally, the tibial nerves sectioned, and electrodes placed on each peroneal nerve. Upon electrical stimulation of the nerve, activity of the anterior compartment of muscles of the lower leg was set up to be recorded on a kymograph as dorsiflexion of the ankle joint. The upper legs were rigidly fixed by means of pins passed through the lower ends of the femora. Interference with circulation was avoided.

Electric contact of the positive electrode over the lower legs (hair clipped off) was by means of gauze pads soaked in a test solution and covered with a metal strip. The negative electrode was a large saline-soaked gauze pad placed over the abdomen (likewise with hair clipped off). The test solution containing d-tubocurarine‡ was made up to a concentration of 143 mg % in 50% ethyl alcohol containing 1:20,000 adrenalin. Alcohol was used because of the evidence that alcoholic solutions favor electrosmosis of positive ions through the skin,[3] and adrenalin because it was hoped that by sub-papillary vasoconstriction it would minimize removal of d-tubocurarine by the blood stream.[4] In 4 experiments the pH of this solution was approximately 4, and in 2 it was adjusted to approximately 7, to favor electroendosmosis through the epidermis.

The experimental plan was to utilize one leg as a test leg, the other as a control. Two types of control were employed. In the first type (4 observations) 15 ma. of direct current was passed through each leg for 20 minutes, but the solution over the control leg contained no d-tubocurarine, only alcohol, adrenalin and one drop of 0.85% NaCl. In the second type (2 observations) solutions containing d-tubocurarine were placed over both legs, but current was passed through only one. Thus, in the first type of control the only variable between the two legs was the presence of d-tubocurarine; in the second type the variable was the passage of current.

Using an electronic stimulator[5] which afforded graded intensity of stimuli of constant duration, the thresholds to a single shock and tetanizing stimuli (50 per second) were determined by recording the response on the kymograph immediately before the passage of the direct current through the legs and

‡ We are indebted to the Abbott Laboratories, North Chicago, Ill., for a supply of this material.

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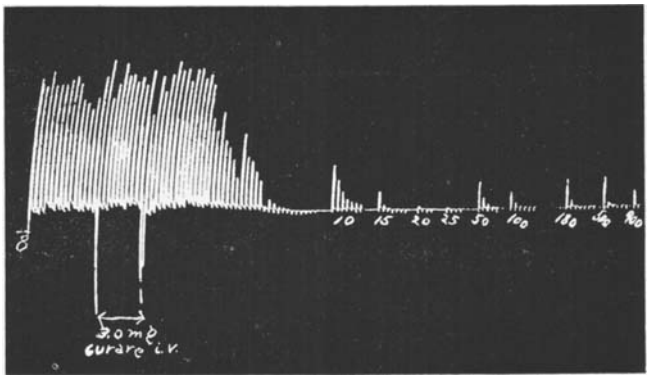

FIG. 1.
Effect of an intravenous curarizing dose upon threshold value of single electrical shocks in a nerve-muscle preparation.
Time scale = one stimulus per second. Regular stimulus at threshold (8 units) was begun, curare was injected and strength of stimulus increased as response fell (all indicated in subscript figure on tracing).

again immediately after the completion of flow of this current for a period of 20 minutes.

Threshold values to a single electric shock or tetanic stimuli remained unaltered in the control leg both after the simple application of d-tubocurarine wet dressings and electrophoresis with a d-tubocurarine-free solution. Similarly, the test leg showed no demonstrable change in thresholds following electrophoresis with the d-tubocurarine solution (Table I). Thus, within the limits of our method, such treatment does not introduce detectable amounts of the drug. It remains to measure the order of magnitude of tubocurarine in muscle that can be detected by our method.

A separate experiment using intravenous d-tubocurarine indicated that the presence of minute amounts of tubocurarine could be readily detected in the muscle. Fig. 1 illustrates the extreme degree of loss of excitability to electrical stimulation of our nerve-muscle system in a 3 kg rabbit receiving 3.0 mg of tubocurarine intravenously. Assuming a total muscle weight of 1.35 kg and 5 g as the weight of the muscles actually tested, then a maximum of 0.011 mg of tubocurarine reached the muscle. This value is an overestimation, since some of the drug injected must remain in the body fluids outside the muscles. Thus, our method is sensitive to less than 0.011 mg of tubocurarine.

Simple calculation (on the basis of electrochemical equivalents) shows that the passage of 15 ma for 20 minutes represents the ionic transfer of 0.1865 m.eq. of a monovalent positive ion or 58.2 mg in terms of tubocurarine.§ Since less than 0.011 mg (0.000035 m.eq.) of the drug can be detected by our method, to curarize our preparation by electrophoresis less than 1/5300 of the current need be transported by the tubocurarine ion, once it has crossed the skin barrier. Our results indicate that even such small amounts could not be introduced by the electric current. Thus, it appears that penetration of tubocurarine, to the muscle layer, if it occurs at all, yields concentrations too minute to be physiologically active.

Summary and conclusions. The question of the effective depth of percutaneous penetration of substances introduced by direct galvanic current was explored, utilizing the effect of d-tubocurarine upon muscle as an indicator system.

Observations on 6 rabbits indicated that threshold values to electrical stimulation of a suitable nerve muscle preparation was not altered after electrophoresis with d-tubocurarine.

Curarization of a rabbit with an intraven-

§ 1 m.eq. tubocurarine = 312 mg.

ous dose revealed that to achieve an effect with tubocurarine electrophoresis only a maximum of 0.011 mg tubocurarine need reach the muscle.

Thus, further evidence is adduced that penetration of substances through the skin by electrophoresis is limited.

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In vitro Effect of Certain Antibacterial Agents on Organisms Encountered in Bovine Mastitis.*

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In screening materials to learn which gave promise in the treatment of bovine mastitis, the *in vitro* effect of a number of antibiotic substances and sulfonamides was determined on some of the bacteria frequently associated with cases of bovine mastitis.

Some information is already available in the literature relative to the materials employed. Salle and Jann[1] reported that subtilin was bacteriostatic in high dilutions and bactericidal in lower dilutions for gram positive organisms, including *Mycobacterium tuberculosis*. Buggs and co-workers,[2] working with streptomycin *in vitro* found a great range in susceptibility of different strains of the same organism.

Goetchius and Lawrence[3] found that 3'-5' dibromo sulfanilanilide *in vitro* was effective in high dilution against *Streptococcus agalactiae, Streptococcus viridans,* and *Brucella abortus*. Schweinberg and Yetwin[4] stated that sulfamethazine *in vitro* was more bactericidal and bacteriostatic than sulfadiazine or sulfamerazine against *Eberthella typhosa, Escherichia coli,* and certain Salmonella. Francis *et al.*[5] reported that 4, 4' diaminodiphenyl sulfone (hereafter designated sulfone) showed promise against *Str. agalactiae* infections in the chick embryo and mouse. Burbaum and others[6] found that sulfadiazine was slightly inhibitory against *Corynebacterium diphtheriae.*

Methods. The organisms used in these experiments were *Str. agalactiae, Br. abortus, Pseudomonas aeruginosa, E. coli,* a coagulase-positive *Staphylococcus aureus,* and *Corynebacterium pyogenes. Salmonella typhimurium* was employed as a representative of the Salmonella.

A culture of each of the above organisms was first grown in tryptose broth for 24 hours at 37°C. Inocula for the actual tests were prepared from such broth cultures. The *C. pyogenes* broth culture was used undiluted while the others were diluted 1 to 100 in broth. Various concentrations of each of the antibacterial agents were prepared in 10 ml of sterile skimmed milk to which brom cresol purple was added as an indicator, and these tubes were inoculated with 0.2 ml of the suspensions of the organisms. Those cultures producing no visible change in milk were streaked on blood agar or nutrient agar slants to determine the extent to which the culture had grown in the presence of the agent. Thus the tests were carried out in skimmed milk

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