bactericidal concentrations varied widely, from 20 mg % for sodium sulfapyridine after 7 days to more than 1000 mg % for sodium sulfadiazine; the bactericidal concentrations of sulfapyrazine and sulfapyridine were consistently lower for the two strains than were the other sulfonamides; sulfacetamide was bactericidal in low concentrations for one of the Sonnei strains (Ch); sodium sulfadiazine was not bactericidal for either Sonnei strain in a concentration of 1000 mg %.

Comparison of the bactericidal concentrations of the 6 sulfonamides for the 3 resistant strains shows that: None of these 3 strains were resistant to sulfapyrazine; that both Sonnei resistant strains were, in addition, not more resistant than the parent strains to sulfacetamide; that the Flexner resistant strain was most resistant to sulfacetamide, sodium sulfadiazine and sodium sulfathiazole; that the 2 resistant Sonnei strains were most resistant to sodium sulfadiazine and sodium sulfathiazole.

Bacteriostatic concentrations increased with time of observation while bactericidal concentrations either decreased or remained the same. Bactericidal concentrations became stabilized much earlier than did the bacteriostatic concentrations.

Summary. Bacteriostatic and bactericidal concentrations of 6 sulfonamides *in vitro* after 2, 4 and 7 days for a resistant strain of *Shigella paradysenteriae* Flexner and 2 resistant strains of *Shigella sonnei* show that: The 3 resistant strains were not resistant to sulfapyrazine; the Sonnei (Ch) strain was in addition, not resistant to sulfacetamide; the Sonnei (Ma) strain was also not resistant to sulfacetamide according to the bactericidal concentrations, but as judged by the bacteriostatic concentrations was more resistant to sulfacetamide than was its parent strain; these 3 strains were quite resistant to the other sulfonamides.

14040

Choleretic Effect of Chloroacetylcholine.

JACK K. FINNEGAN AND G. A. EMERSON.
From the Department of Pharmacology, West Virginia University School of Medicine, Morgantown.

A choleretic response to monochloroacetic acid was first noted by Chabrol *et al.*[1] and has been confirmed by one of us with J. L. Morrison. Data concerning this agent will be reported elsewhere. Of interest here is the singular latency of choleresis following intravenous injection of chloroacetate; the maximum response is usually delayed for 45-90 min. This latency indicates that perhaps chloroacetic acid does not act as a direct chloretic but through the slow formation of some more potent metabolite. Chloroacetate may be converted to glycolate in the body.[2] However, glycolate is not an active choleretic even when given in large doses intravenously. Similarly, mercaptoacetate and aminoacetate do not significantly stimulate formation of bile. An opportunity to test another "physiologic" derivative of chloroacetate, chloroacetylcholine, was afforded through the kindness of Dr. Morrison and Dr. David Fielding Marsh, who supplied us with a small amount of the chloride of this ester. Other pharmacologic actions of chloroacetylcholine have been recently described.[3]

The technic used was similar to that employed previously in noting the response to chloroacetate in some 20 dogs. A large male dog was lightly narcotized with pentobarbital and received supplemental amounts of

1 Chabrol, E., Charonnat, R., Maximin, M., and Waitz, R., *Compt. Rend. Soc. Biol.*, 1931, **106**, 17.

2 Morrison, J. L., *Comparative Biological Effects of Halogenated Fatty Acids*, Ph.D. diss., 1941, Univ. of California.

3 Morrison, J. L., to be published.

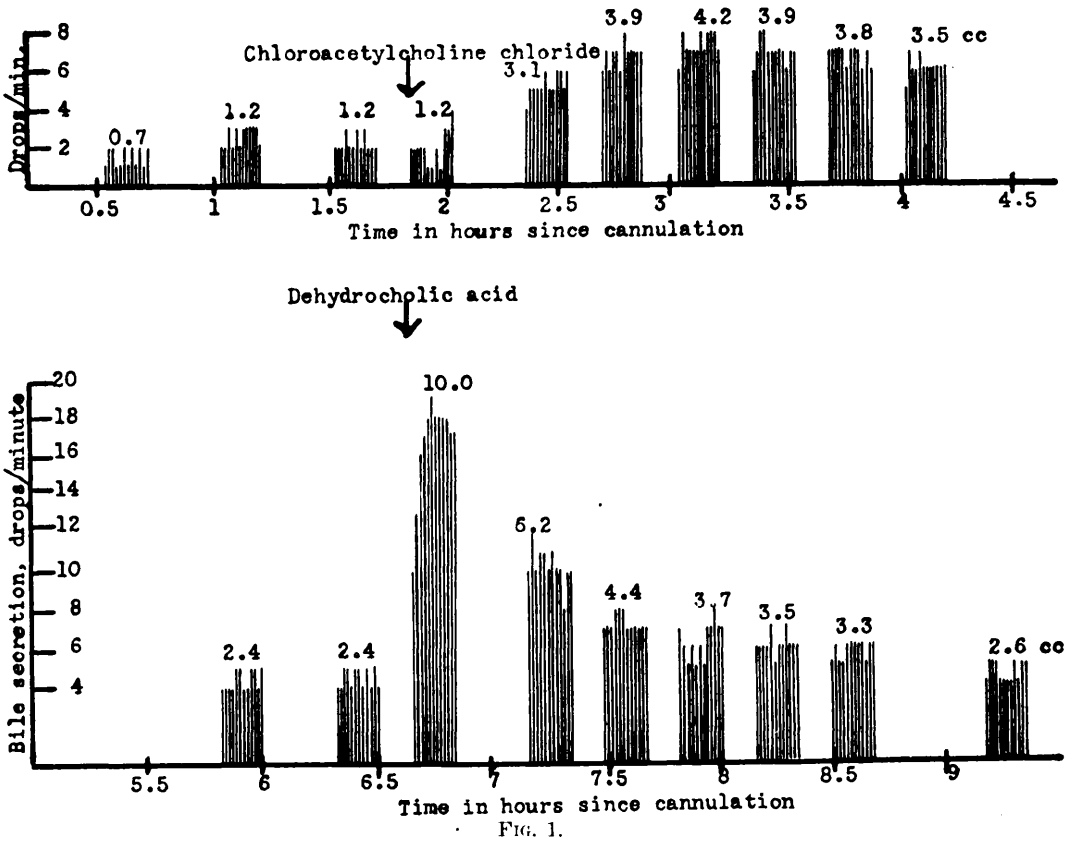

FIG. 1.
Male Dog. 36 Kg.
Comparison of choleretic effects of 20 mg/kg of chloroacetylcholine and dehydrocholic acid.

barbital. The common bile duct was cannulated and the cystic duct was clamped off with a hemostat after the entire contents of the gallbladder had been expressed through the cannula. The cystic bile was then injected into the duodenum to insure an adequate initial flow of hepatic bile to flush the cannula. Observations of the number of drops of bile were recorded manually through a signal magnet writing upon a synchronous kymograph and were begun 30 min after the operative procedure. The volume of bile secreted was measured at 12 min intervals. Injection of chloroacetylcholine into the femoral vein was made after 2 uniform control periods of observation. The integrity of the block of the cystic duct and the patency of the cannula in the common duct were tested at necropsy.

Carotid pressure was recorded continuously and did not fall below 130 mm Hg during the observations except for a brief fall of 55 mm immediately following injection of chloroacetylcholine. The choleretic effects were independent of changes in systemic blood pressure.

Results are illustrated in Fig. 1, in which a comparison is made of the choleretic effects of 20 mg/kg of chloroacetylcholine chloride and a similar quantity of the Na salt of dehydrocholic acid (decholin) after intravenous injection. Bile flow is represented on the ordinate as drops/min with time intervals on the abscissa. Each group of lines thus represents the number of drops of bile secreted per min for each of 12 consecutive min. The maximum response to chloroacetylcholine did not occur until more than 1 hr after injection while the maximum response to decholin was immediate. That the increment in flow after chloroacetylcholine was not an artifact due to presence of cystic

bile in the duodenum is indicated by the time of response, 3.5 hr after introduction of bile into the duodenum, and by observations on 3 control dogs not given chloroacetylcholine. In these dogs, as in several others given glycine or other inactive agents, a latent response was not observed.

The volume of bile secreted during each 12-min period is also noted on the graph. At the time of maximum response, the flow of bile following injection of chloroacetylcholine was 350% of the control level, and after decholin it was 417% of the corresponding control level. Since the control level of secretion before decholin was given was higher than the initial control level, decholin must be considered to be relatively more potent than these values indicate.

Major differences were observed in the color and specific gravity of bile secreted under the influence of the 2 agents. After injection of chloroacetylcholine, the specific gravity increased considerably and attained a peak at the time of maximum flow, while the color was unchanged, indicating a true choleretic effect. After decholin, the usual hydrocholeresis was noted; the bile became pale and the specific gravity fell below the control level of 1.012.

The similarity of the delayed choleretic response to chloroacetate and chloroacetylcholine indicates that the latter rapidly liberates chloroacetate in the body, which then reacts slowly to evoke choleresis. This view is in accord with the hypothesis that a free carboxyl group is essential for the choleretic action of halogenated hydrocarbon derivatives,[1] and the evanescence of other pharmacologic effects of chloroacetylcholine also indicates rapid hydrolysis in the body.[3] Chloroacetylcholine appears to cause a stronger choleretic response than equivalent amounts of chloroacetate, but this may be due to a greater hepatotropism of the ester as compared to the free acid, which is probably distributed as uniformly throughout the tissues as is tribromoacetate.[4] The cholinergic properties of the ester are unrelated to its choleretic action, because of the difference in time of the 2 types of response and also because of the general lack of choleretic effect of other cholinergic agents.[5]

Summary. Chloroacetylcholine chloride provokes a large but delayed increase in the formation of bile in the dog, similar to the choleresis following administration of chloroacetate. The comparative choleretic effects of the 2 agents are discussed in regard to the mechanism of chloroacetate choleresis.

4 Emerson, G. A., and Morrison, J. L., *J. Pharm.*, 1942, **75**, 226.

5 McGuigan, H. A., *Applied Pharmacology*, Mosby, St. Louis, 1940.

14041 P

Phosphocreatine as Energy Source of the Action Potential.

D. NACHMANSOHN,* R. T. COX, C. W. COATES, AND A. L. MACHADO. (Introduced by Fred A. Mettler.)

From the Department of Neurology, College of Physicians and Surgeons, Columbia University, the Department of Physics, New York University, and the New York Aquarium.

The parallelism between the concentration of choline esterase and the E.M.F. of the action potential in electric organs and the localization of the enzyme at the neuronal surface suggest that formation of acetylcholine is intrinsically connected with the nerve action potential and may be responsible for the electrical changes during nerve activity.[1-3]

* Aided by grants of the Dazian and Josiah Macy, Jr., Foundations.

1 Nachmansohn, D., and Meyerhof, B., *J. Neurophysiol.*, 1941, **4**, 348.

2 Nachmansohn, D., Cox, R. T., Coates, C. W., and Machado, A. L., *J. Neurophysiol.*, 1942, **6**, 499.

3 Nachmansohn, D., and Steinbach, H. B., *J. Neurophysiol.*, 1942, **5**, 109.