butylamino) group (W243).

It remains to be seen whether acriflavine acts in the same manner as the nitroacridines. The active constituent of acriflavine seems to be the 10-methyl acridinium chloride (see Mauer[1]). Proflavine, which is the sulphuric acid salt without the 10-methyl group, was much less inhibitory for the feline pneumonitis agent in chick embryos. Its activity against other viruses of the group is under investigation.

The relative resistance of the mouse pneumonitis virus to acriflavine and to one of the nitroacridines is surprising in view of the high sensitivity of this agent to penicillin[13,14] and sulfonamides.[15] The feline pneumonitis and meningopneumonitis viruses which are, on the other hand, relatively resistant to penicillin and are not affected in mice or chick embryos by sulfonamides are quite readily inhibited in chick embryos by acriflavine and the nitroacridines. The observed differences in the chemotherapeutic spectrum for penicillin, sulfonamides, and acridines suggest interesting variations in the enzymatic or metabolic constitution of the agents of the psittacosis-lymphogranuloma group. The 3 classes of substances probably act on 3 different constituents of the viruses under consideration.

The results in mice tended to parallel those in chick embryos, but on a lower scale of activity so that less conclusive results were obtained. Effective inhibition of respiratory infections was observed only with the 2 nitroacridines against the cat pneumonitis agent, but these 2 substances were, on a weight basis, more effective than penicillin against this virus. Because of the high toxicity of acridines and their failure to show general activity against the psittacosis-lymphogranuloma group in mice, no claim for their therapeutic usefulness can be made at the present time.

*Summary.* Acriflavine; 3-nitro-6, 7-dimethoxy 9 - (2-phenyl-4-diethylaminobutylamino) acridine; and 3-nitro-6, 7-dimethoxy 9 - (2-hydroxy - 3-diethylaminopropylamino) acridine inhibited yolk sac infections of chick embryos with the agents of feline pneumonitis, lymphogranuloma venereum, and meningopneumonitis. The first two compounds were less active against the virus of mouse pneumonitis, but the last-named inhibited this agent in chick embryos.

Proflavine, atabrine, and drugs closely related to the above-mentioned nitroacridines, except for substitution of Cl for $NO_2$, had no significant inhibitory action. 3-nitro-9-aminoacridine was intermediate in its effect.

Respiratory infections in mice caused by the agent of feline pneumonitis were retarded by the two nitroacridines, but these drugs showed slight or no effect in mice against intranasal infection with the agents of mouse pneumonitis, lymphogranuloma venereum, and meningopneumonitis.

12 Horsfall, F. L., Jr., *J. Exp. Med.*, 1939, **70**, 209.

13 Meiklejohn, G., Wagner, J. C., and Beveridge, G. W., *J. Immunol.*, 1946, **54**, 1.

14 Eaton, M. D., Dozois, T. F., van Allen, A., Parish, V. L., and Schwalm, S., *J. Immunol.*, in press.

15 Eaton, M. D., and Hanford, V. L., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 63.

## 16012

## Effect of Egg Yolk on Release of Antigen from Rickettsiae.

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The existence of a soluble antigen which is released on treatment of certain rickettsiae with ether has been well established with respect to *Rickettsia prowazeki*,[1] *R. rickettsii*,[2] and *R. akari*.[3,4] That this antigen, in the case

1 Topping, N. H., and Shear, M. J., *Pub. Health Rep.*, 1944, **59**, 1671.

of typhus fever rickettsiae at least, is complete is shown by its activity both *in vitro* and *in vivo*. *In vitro* it is active as a complement fixation test antigen[1] and a precipitin test antigen,[5] and *in vivo* it is able to immunize guinea pigs against the disease[1] with the formation of antibodies demonstrable in the complement fixation test,[1] neutralization and precipitin tests.[6] By the use of the electron microscope, it has been demonstrated that the antigen probably arises from a capsule-like structure on the surface of the organism, and that it exists as a suspension of submicroscopic particles of the capsular substance.[7]

When purified suspensions of rickettsiae were prepared without the use of ether and subsequently shaken with ether[10] little soluble antigen could be demonstrated. When a normal yolk sac suspension was added to the purified rickettsiae, soluble antigen was released with ether. Further experiments are presented here to show that some component of egg yolk plays a part in facilitating the release of the soluble antigen from rickettsiae by ether treatment at room temperature.

The rickettsial suspensions used in these experiments were prepared from infected yolk sacs of chicken embryos. By differential centrifugation efforts were made to remove some of the yolk sac tissues and soluble proteins. The rickettsiae were finally suspended in a limited quantity of saline or distilled water containing 0.1% formalin. In the antigen release experiments generally 0.2 ml of the rickettsial suspension was mixed with 0.6 ml of diluent, either the universal buffer of

TABLE I.

Effect of Normal Egg Yolk on Release of Antigen from Rickettsiæ. Homologous convalescent guinea pig serum was used in each instance.

| Soluble antigen from | Conc. of soluble antigen (as original yolk sac) % | pH | Diluent | Complement fixation results Dilutions of soluble antigen 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | Antigen control 1:4 |
|---|---|---|---|---|---|---|---|---|---|---|
| *R. prowazeki** | 25 | 6.14 | 2.5% yolk | ++++ | ++++ | ++++ | ++ | — | — | — |
| Breinl strain) | | 6.10 | Buffer only | +++ | — | — | — | — | — | — |
| *R. mooseri** | 15 | 6.1 | 2.5% yolk | ++++ | ++++ | ++++ | ++++ | ++ | — | — |
| (Wilmington strain) | | 6.0 | Buffer only | + | — | — | — | — | — | — |
| *R. rickettsii*† | 50 | 6.2 | 2.5% yolk | ++++ | +++ | + | — | — | — | — |
| (Bitter Root strain) | | 5.6 | Buffer only | ++ | trace | — | — | — | — | — |
| *R. akari*‡ | 25 | 6.0 | 2.5% yolk | ++++ | ++++ | ++++ | trace | — | — | — |
| (Wild mouse strain) | | 5.95 | Buffer only | ++++ | ++++ | trace | — | — | — | — |
| *R. burneti*† | 50 | 6.14 | 2.5% yolk | — | — | — | — | — | — | — |
| (Italian strain) | | 6.10 | Buffer only | — | — | — | — | — | — | — |

* Rickettsiæ were washed with salt solution pH 7.6 and distilled water according to the method of Shepard and Topping.[10]
† Rickettsiæ were sedimented once, then resuspended in saline.
‡ Rickettsiæ were sedimented two times and resuspended in distilled water.

2 Plotz, H., Reagan, R. L., and Wertman, K., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 173.

3 Huebner, R. J., Stamps, P., and Armstrong, C., *Pub. Health Rep.*, 1946, **61**, 1605.

4 Huebner, R. J., Jellison, W. L., and Pomerantz, C., *Pub. Health Rep.*, 1946, **61**, 1677.

5 Shepard, C. C., and Topping, N. H., *Nat. Inst. Health Bull., No. 183*, Government Printing Office, Washington, 1945, p. 87.

6 Shepard, C. C., *Nat. Inst. Health Bull. No. 183*, Government Printing Office, Washington, 1945, p. 93.

7 Shepard, C. C., and Wyckoff, R. W. G., *Pub. Health Rep.*, 1946, **61**, 761.

Michaelis[8] or the same buffer containing 2.5% of normal yolk from fresh chicken eggs. To each mixture were added 1.5 ml of anaesthetic ether. The two phases were then shaken together vigorously 30 times. The aqueous layer was drawn off, freed of ether with vacuum and centrifuged at 4000 r.p.m. for one hour to sediment the rickettsiae. The supernatants were tested for antigen content by the complement fixation test[9] using 4 units of homologous convalescent guinea pig serum. The results of the tests with five species of rickettsiae are shown in the table.

The readings in the table show that in the presence of 2.5% yolk higher titers were obtained with *R. prowazeki, R. mooseri, R. akari,* and *R. rickettsii.* With *R. burneti* no soluble antigen could be detected.

When normal yolk was diluted 10 times with 0.85% sodium chloride solution and extracted with 2 volumes of ether, it was found that the water soluble constituents in the aqueous layer had the same degree of activity in releasing soluble antigen from rickettsiae as the original yolk.

Although it will be noted that some antigen was released without the addition of yolk, such release may have been due to the amount of yolk remaining with the preparation of rickettsiae, since the more carefully purified Breinl and Wilmington suspensions showed little release except when yolk was added. It has been noted that the increase in titer obtained by adding yolk is variable unless the rickettsiae are carefully washed. Yolk sacs are known to carry more or less yolk after harvest and it is evident that the amount of adherent yolk can effect the complement fixation titer of an ether extracted antigen. For consistent results it has been found advantageous to add 5 or 10% normal egg yolk to all method I and method II antigens[11] for use in complement fixation tests.

In the preparation of Rocky Mountain spotted fever antigens by method I or II,[11] the addition of yolk is especially valuable[12] since the growth of *R. rickettsii* in yolk sacs is poor, and antigen release is frequently not marked.

*Summary.* The addition of as little as 2.5% egg yolk to suspensions of yolk sacs infected with the rickettsiae of epidemic and endemic typhus fever, Rocky Mountain spotted fever, and rickettsialpox (*R. prowazeki, R. mooseri, R. rickettsii,* and *R. akari*) prior to treatment with ether has resulted in antigens with enhanced titers as measured by the complement fixation test.

8 Michaelis, L., *Biochem. Z.*, 1931, **234**, 139.

9 Bengtson, I. A., *Pub. Health Rep.*, 1944, **59**, 402.

10 Shepard, C. C., and Topping, N. H., *J. Immunol.*, 1947, **55**, 97.

11 Topping, N. H., and Shepard, C. C., *Pub. Health Rep.*, 1946, **61**, 701.

12 Unpublished experiments.

## 16013

## Renal Hemodynamic Effects of Adrenaline and "Isuprel": Potentiation of Effects of Both Drugs by Tetraethylammonium.

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Vasodepressor drugs have been prepared which are built on the chemical nucleus of sympatheticomimetic compounds. The pharmacological properties of one of these, the N-isopropyl homologue of adrenaline, called "Isuprel" (1-(3[1],4[1]-dihydroxyphenyl)-2-isopropylaminoethanol hydrochloride) have been described by Lands and his colleagues.[1] The suggestion has been made that the activity of

1 Lands, A. M., Nash, V. L., McCarthy, H. M., Grainger, H. R., and Dertinger, B. L., *J. Pharm. Exp. Therap.*, 1947, **89**, 110.