

in contributing to the effectiveness of insecticides.

3. The approximate LD₅₀ for γ 666 applied to the body surface is 0.4 mg per kg for the newly emerged fly (*Musca domestica*). For the older adult the LD₅₀ is about 0.8 mg per kg for *Musca domestica* and 0.6 mg per kg for *Calliphora spp.* This decrease in sensitivity as the fly ages is less than that reported for DDT.

4. Gamma 666 is about twice as toxic as DDT for the cockroach (*Periplaneta americana*), and is distinctly more toxic than DDT for *Musca domestica* (newly emerged and adult) and *Calliphora spp.* (adult).

5. Both death and knockdown occur more rapidly after surface application of γ 666 to *Musca domestica* and *Periplaneta americana* than after DDT.

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Type-specific Capsular Swelling of Meningococci by Chicken Antiserum.

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Branham¹ has recently emphasized the value of serologic typing of meningococci for epidemiologic studies as well as for more exact etiologic diagnosis. The methods currently employed for this purpose include (a) capsular swelling with hyperimmune rabbit serum and (b) agglutination with monovalent antisera prepared in rabbits or chickens. One of the advantages of using chickens as a source of agglutinating serum is their ability to tolerate doses of meningococci which may be toxic or even lethal for rabbits.^{2,3}

During the past 18 months we have had occasion to prepare such antisera in adult chickens. The course of injections was based on the report of Phair, Smith and Root.² Doses of 2 to 4 billion living organisms, derived from casein-hydrolysate starch agar cultures⁴ and suspended in physiological saline, were given intravenously at intervals of approximately one week. After 4 injections

the animals were allowed a rest period of 7 to 16 days before being bled; in some instances this bleeding was followed after 7 to 9 days by a fifth injection and the animals were kept 1 to 4 weeks longer before final exsanguination. The serum collected from the successive bleedings of each chicken was pooled and stored at 2°C. Throughout the period of immunization the birds appeared healthy and showed no appreciable loss of weight.

In testing for agglutinins, meningococci from cultures grown 5 to 18 hours on casein-hydrolysate starch agar were suspended in saline to a concentration of 2 to 4 billion cocci per ml. Dilutions of antiserum were mixed with an equal volume (0.3 ml) of culture suspension in small tubes and these were agitated vigorously on a Kahn shaking machine for 10 to 20 minutes at room temperature before readings were made. Agglutination was readily visible with the naked eye. The titer of the serum was recorded as the highest dilution, after addition of antigen, in which clumping was marked. The results summarized in Table I show that following immunization of chickens with 16- to 18-hour cultures or with cultures only 4 hours old, we obtained satisfactory agglutinating sera versus strains of meningococci

¹ Branham, S. E., *Am. J. Pub. Health*, 1945, **35**, 232.

² Phair, J. J., Smith, D. G., and Root, C. M., *Proc. Soc. Exp. Biol. and Med.*, 1943, **52**, 72.

³ Miller, C. P., *Yale J. Biol. and Med.*, 1944, **16**, 519.

⁴ Mueller, J. H., and Hinton, J., *Proc. Soc. Exp. Biol. and Med.*, 1941, **48**, 330.

TABLE I.
Summary of Data on Immunization of Chickens with Meningococci.

Animals employed			Immunizing antigens			Type-specific serologic properties of antiserum	
No.	Breed	Sex	Serologic type	Strain	Age of culture (hr)	Capsular swelling	Agglutination titer
68	Rhode Island Red	♀	I	1027	16-18	+	120
1	Plymouth Rock	♂	I	1027	4	+	240
7	White Rock	''	I	1027	4	+	120
66	Rhode Island Red	♀	II	963	16-18	0	80
49	'' '' ''	''	II α	1054	16-18	+	40
3	Buff Orpington	♂	II α	1054	4	+	160
8	White Rock	''	II α	1054	4	+	320

Types I, II and II alpha, as others have done.^{2,3,5}

Investigation was then made to determine whether these agglutinating sera would also exhibit the property of capsular swelling as is the case with rabbit anti-meningococcal sera of adequate potency. Suspensions of living organisms from 4- or 5-hour cultures on Mueller-Hinton medium were mixed in the usual fashion with a loopful of chicken antiserum plus a loopful of methylene blue stain. Microscopic observation revealed prompt and strikingly clear-cut capsular swelling of meningococci Types I and II alpha in the presence of their homologous antisera; no capsular swelling was demonstrable when cocci of these serologic types were mixed with any heterologous antiserum. No capsular swelling was seen in the mixtures of Type II meningococci with the single homologous serum available or with the several antisera versus the other 2 types. The above findings have been checked and found to hold true not only for the various sera as freshly drawn but also after storage at 2°C for

periods from 8 to 14 months. The data in Table I also indicate that sex or breed does not markedly affect the success of immunization providing healthy adult birds, weighing 5 to 7 pounds, are employed.

Discussion. So far as we are aware, data on the use of chickens for the preparation of specific capsular swelling antisera against meningococci or other bacterial species have not been previously reported. The present findings are of interest in demonstrating that capsular swelling may be produced with antisera derived from animals other than the rabbit and the horse; they also suggest that chickens can be used to prepare quellung sera for bacteria to which the rabbit or horse may not respond favorably.

From a practical standpoint, the ease with which adult chickens can be successfully immunized for the production of quellung antiserum versus meningococci Types I and II alpha should make it feasible for many diagnostic laboratories to prepare a supply. Such sera can undoubtedly be used for rapid and direct serologic identification of meningococci in the spinal fluid as well as in cultures obtained from other sources.

⁵ Kabat, E. A., Miller, C. P., Kaiser, H., and Foster, A. Z., *J. Exp. Med.*, 1945, **81**, 1.

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The Activity of Anionic Surface Active Compounds in Producing Vascular Obliteration.

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The great variety of substances used for obliteration of varicose veins have the common property of being destructive to intima of the vein.^{1,2} Some of the substances are