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Quinine Hypersensitivity in Guinea Pigs.

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In an attempt to widen the range of compounds amenable to experimentation on hypersensitiveness in animals, using substances which may differ in the mechanism of sensitization, we undertook to sensitize guinea pigs with quinine which is known to cause allergy in human beings. Some positive sensitization results of moderate degree were obtained after a number of applications to the skin of a 5% solution in a mixture of one part of butyl carbitol (diethylene glycol monobutyl ether) and 3 parts olive oil. In several experiments in which this treatment was made instead on inflamed areas the number and degree of positive effects were increased; inflammation was produced with cantharidin, similar to previous work,[1] along with other irritants (xylene, croton oil, etc.). Likewise, marked sensitivity was achieved when quinine was applied to areas inflamed by 2:4 dinitrochlorobenzene in animals sensitive to the latter substance.

In well sensitized animals the reactions following the application of one drop of the above quinine solution are well developed by the following day and consist of intense reddening and slight elevation of the treated area. In less sensitive animals pronounced reactions appeared after 2 or 3 consecutive daily applications to the same site.

In a preliminary experiment the method of cantharidin burns gave positive sensitization effects also with a nickel salt (sulfate).

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Immunizing Value of Rickettsial Vaccines.

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Recently developed methods for the cultivation of Rocky Mountain spotted fever and typhus rickettsiae in agar slant tissue cultures[1]

[1] Landsteiner, K., and DiSomma, A. A., *J. Exp. Med.*, 1940, **72**, 361.

[1] Zinsser, H., Wei, H., and Fitzpatrick, F., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 604; *J. Exp. Med.*, 1939, **69**, 179.

and in the yolk sac[2] of the developing chick embryo have opened new possibilities for the preparation of vaccines against these infections. We have been making comparative studies (a) of the immunizing potencies of vaccines obtained from rickettsiae grown in these ways and (b) of the relative merits of these procedures for the large scale production of vaccines.

These comparative tests have been made using an "eastern" strain of spotted fever rickettsiae of relatively low virulence. It had been maintained in agar tissue cultures for at least one year before being used in the present experiments. It was uniformly infectious for guinea pigs but unlike more virulent strains of spotted fever the mortality rate among diseased pigs was not high. In spite of this difference in virulence vaccines made from it protected guinea pigs against many lethal doses of "western" spotted fever rickettsiae, and vice versa.

For the production of vaccines[3] this rickettsial strain was carried both on agar slant cultures and in developing eggs. When grown in culture large test tubes containing Tyrode-serum agar slants were inoculated with infected tissue according to the technic described by Zinsser, Wei and Fitzpatrick.[1] The tubes were incubated at 35°C for 5, 6, or 7 days. At the end of this period smears were made from bits of tissue detached from the agar slants, strained and examined for rickettsiae. Cultures showing good growth of rickettsiae were harvested by scraping off the tissue with a small spatula, care being taken not to disturb the agar surface itself. This was then used immediately for the production of vaccine. It was early observed that many rickettsiae could be demonstrated in smears prepared by scratching the surface of the agar slants after this removal of tissue; and it was thought that several crops of infected tissue might be obtained from the same slant by merely reseeding the harvested tube with fresh embryonic pulp. This was the case. Therefore, after harvesting its infected tissue a slant was allowed to absorb carbon dioxide to neutralize the acidity it had developed as a result of the previous tissue metabolism; it was then seeded with fresh pulp and incubated to produce a new crop of infected tissue. This was continued as long as the rickettsial yield remained high and no bacterial contamination occurred, as shown by microscopic examination made at the end of the incubation period. Usually a slant was reinoculated 3 or 4 times in this fashion before being discarded. This process of reinoculation reduced very materially the cost of vaccine production, both in

[2] Cox, H. R., *U. S. Public Health Rep.*, 1938, **53**, 2241; 1939, **54**, 1070.

[3] Zinsser, H., Plotz, H., and Enders, J. F., *Science*, 1940, **91**, 51.

time and in cost of nutrient medium. To make vaccine the infected tissue taken from the agar slants was weighed and thoroughly ground. A phosphate-saline buffer solution of pH 7.0 containing 0.25% formalin and 0.25% phenol was added to this mass in an amount sufficient to make a 2% suspension of tissue. The buffer solution was 0.05M with respect to phosphate ions and 0.15M with respect to NaCl. Crude vaccine thus prepared was inactivated by being kept for 48 hours at 70°F and for at least 5 days in the chill room before use. At the conclusion of the inactivating period it was lightly centrifuged to remove any large clumps of tissue that remained. About 10 liters of Zinsser-type vaccine were made in this fashion in 5 separate batches. Samples of each of these were separately tested for potency in the comparative tests to be described below.

The procedure used in preparing vaccine according to the yolk sac method of Cox was as follows: Fertile eggs which had been incubated for 7 to 8 days were inoculated by injecting 0.5 cc of highly infectious rickettsial suspension directly into the yolk sac of each egg. Nearly all embryos were moribund in from 3 to 4 days. Microscopic study of smears of yolk sac tissue from such embryos showed a considerable number of rickettsiae with smaller numbers in smears of the chorioallantoic membrane. Vaccine has been made from the combined yolk sac and chorioallantoic membranes taken from moribund embryos. In harvesting them these membranes, freed from the embryos and other egg contents, were placed on sterile wire mesh and excess of yolk was drained off under mild pressure. The infected membrane tissue was then weighed and thoroughly ground. Saline solution containing 0.3% formalin and 1% phenol was added to this ground mass in the proportion of 100 cc to every 10 g of tissue. This concentrated suspension was inactivated by being left for 48 hours at 70°F and 5 days in the chill room. It was then centrifuged for 45 minutes at 2000 r.p.m. The supernatant fluid was separated, filtered through 12 to 15 layers of sterile gauze, measured and diluted with enough saline solution to give the equivalent of a 2% suspension of the original tissue. This was the finished vaccine. Four different lots of vaccine made in this fashion were employed in the comparative potency tests.

The methods used in preparing these Zinsser and Cox type vaccines are similar but not exactly the same. Both were made 2% with respect to the original infected tissue. The centrifugal clarification was much less complete with the Zinsser vaccine; this should have favored its potency by retaining in it any rickettsiae still associated with tissue fragments. The Cox vaccine was inactivated with

strong formalin and phenol; if these antiseptics had a deleterious effect on the antigenicity of the rickettsiae or their products they should have weakened, relatively, the immunizing capacity of the yolk sac vaccine.

The immunizing potencies of all lots of both types of vaccine have been tested in guinea pigs. To do this portions of each lot of vaccine were diluted with from 2 to 16 parts of saline solution. Series of guinea pigs were injected subcutaneously with one cc doses of these diluted vaccines. The vaccination of each animal was completed by giving a second equal injection after one week. Seven days after this second vaccination the immunity of the guinea pigs was determined by injecting them intraperitoneally with one cc of a 1:1000 dilution of infected yolk sac tissue rich in rickettsiae. In every experiment unvaccinated guinea pigs serving as controls were injected with this same virus dose; without exception these pigs developed characteristic fever. On 12 occasions we have made dilution titrations of the infectivity of yolk sac tissue diseased with the strain of spotted fever rickettsiae used in these experiments. The end point was always at the $1:10^6$ dilution so we may conclude that the challenge dose administered in the present experiments was of the order of 1000 guinea pig-infectious doses; no unvaccinated control guinea pig failed to become sick and develop typical fever after the injection of this dose of rickettsiae.

The daily temperatures of typical unvaccinated and vaccinated guinea pigs after challenge inoculation are recorded in Table I. The result of vaccine-protection studies with the 5 lots of Zinsser- and

TABLE I.

Temperatures (°F) of Guinea Pigs after Injection of Challenge Rickettsial Dose.

Days after injection	Guinea Pig No. A Vaccine Dose 0.0cc	B 0.0cc	C 0.0cc	D 0.50cc	E 0.25cc	F 0.125cc	G 0.125cc
1	101.4°	103.0°	101.0°	101.9°	102.4°	102.4°	102.2°
2	120.4	103.3	103.2	100.8	102.2	102.0	101.0
3	102.0	103.6	103.8	100.6	101.6	102.8	100.4
4	103.8	*105.4*	102.9	101.2	101.2	103.0	101.4
5	*105.0*	*105.2*	*104.9*	102.0	102.0	103.0	101.0
6	*105.4*	105.5	*105.0*	102.6	101.6	102.6	100.8
7	104.4	104.6	104.9	101.8	101.4	*103.8*	101.4
8	105.1	104.3	104.4	100.4	101.8	*104.0*	101.4
9	105.3	103.0	104.3	101.6	102.0	104.0	101.0
10	D	104.1	103.8	102.2	102.4	104.0	101.2
11	—	103.5	102.9	102.4	102.6	103.0	101.6
12	—	103.7	102.0	102.6	102.4	103.2	101.8
13	—	D	—	101.8	102.0	S	101.0

Note: In this table D indicates death and S survival. The point of onset of disease has been indicated by italicized daily temperatures. According to our evaluation animals D, E and G were completely, animal F was incompletely protected.

the 4 lots of Cox-type vaccine are collected in Table II. Taking the continued health of the animal and the absence of fever as index of protection it is obvious that all 9 batches of vaccine were able to convey a high degree of protection to guinea pigs. Zinsser vaccine protected all animals when a total of at least 0.25 cc of the original vaccines was administered. When the dose was reduced to 0.125 cc from 50 to 66% of the animals were fully protected. Three lots of Cox vaccine gave complete protection down through a total dose of 0.125 cc, corresponding to the lowest dilution tested. The fourth and weaker lot gave complete protection at 0.25 cc but not at 0.125 cc. From these experiments it would appear that the yolk sac vaccine is at least as effective an immunizing agent for guinea pigs as the tissue culture vaccine. We have made large volumes of vaccine by both methods and are convinced that under the conditions necessary

TABLE II.
Comparative Immunizing Values of Rickettsial Vaccines.

Lot No.	No. of vaccinated animals	Total amt of vaccine, cc	No. of animals showing no fever	% Protection
1Z	4	1.00	4	100
	4	0.50	4	100
	3	0.25	3	100
2Z	4	1.00	4	100
	4	0.50	4	100
	4	0.25	4	100
	3	0.125	2	66
3Z	4	1.00	4	100
	4	0.50	4	100
	4	0.25	4	100
4Z	4	0.25	4	100
	4	0.125	2	50
5Z	4	0.25	4	100
	4	0.125	4	50
1C	4	1.00	4	100
	4	0.25	4	100
	3	0.125	2	66
2C	3	1.00	3	100
	4	0.25	4	100
	3	0.125	3	100
3C	4	1.00	4	100
	4	0.25	4	100
	4	0.125	4	100
4C	2	1.00	2	100
	4	0.25	4	100
	4	0.125	4	100

Note: In this table Z and C refer to vaccines made according to the agar slant and yolk sac methods of Zinsser and Cox respectively.

to produce vaccine in large amounts the yolk sac technic is also easier to carry out and much less costly in time and materials.

Though the foregoing experiments were made with an eastern strain of spotted fever rickettsiae, we have also experimented with more virulent strains of the disease and with the Breinl strain of epidemic typhus rickettsiae. From our experience we are convinced that the conclusions just expressed are equally applicable to problems of making vaccine against the diseases caused by these other rickettsiae.

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Physiological and Toxicological Effects of Some Fish Muscle Extracts.

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While occasional descriptions of poisoning by fishes are encountered in ancient writings, surprisingly few scientific data of experimental nature on this subject are to be found in modern pharmacological literature Of medicinal agents derived from fishes and mentioned in modern materia medica probably the best known is cod liver oil, which physicians used empirically long before discovery of vitamin D. Another "fish" product employed in therapeutics is spermacetti, well known ointment base. With the advent of the vitamin era an intensive search was naturally made for these substances in other fishes than the cod, *e. g.*, in the halibut, haddock and menhaden. Another interesting study of what may be called a drug rather than a toxic agent was that made by Macht and Barba-Gosé on the oil expressed from the flesh and bones of the *Ruvettus pretiosus,* popularly known as the "castor-oil" fish.[1] This investigation was of special interest in respect to the chemical nature of the laxative principles contained in the oil.[2] Other poisons (not of putrefactive origin) are found in various fishes but few researches of an experimental nature have been published concerning them. Here belong the contributions made by Mosso[3] on the toxicity of the

[1] Macht and Barba-Gosé, *J. Am. Pharm. Assn.*, 1931, **20**, 558.

[2] Macht and Barba-Gosé, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 772.

[3] Mosso, *Arch. f. exp. Path.*, 1888, **25**, 111.