starch grains. In the case of the canna clusters, which are composed of finer needles than the potato clusters, the colors were rather purplish and orange instead of blue and gold as in the natural granules.

The observations of the writers are apparently the first on record in which from clear solutions a crystalline precipitate has been obtained, which shows optical properties very similar to those shown by natural starch. While further investigation is required to determine whether or not this crystalline substance is identical with any substance in the natural starch grain, the fact that it behaves as does natural starch when viewed between crossed nicols renders it extremely probable that the "black cross" shown by the natural starch grain in polarized light is due to crystalline structure rather than to strain or lamination.

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Local immunization of guinea pigs to cutaneous infection with a pasteurella isolated from wild rats.

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In the course of the routine plague control work carried on by the U. S. Public Health Service under the direction of Dr. N. E. Wayson[1] a pasteurella infection of wild rats was encountered which seriously interfered with the rapid and accurate diagnosis of chronic rodent plague. As a rule guinea pigs cutaneously infected with the suspected tissues succumbed in 1 to 2 days to the pasteurella disease. In order to rule out mixed infections with *P. pestis* a number of special methods which will be published elsewhere were tried. It was apparent that an immunological procedure capable of protecting guinea pigs rapidly and completely against the pasteurella would probably allow the separation and

[1] Wayson, N. E., *Pub. Health Rep.*, 1925, lx, 1975.

detection of *P. pestis* in a cadaver carrying the hemorrhagic septicemia organism in the spleen, lung, etc. Passive and active immunization of guinea pigs with living avirulent or killed organisms introduced by the subcutaneous and the intraperitoneal route were tried with varying success. A detailed account of these experiments is not in the scope of this preliminary communication but attention should be called to some observations on local immunizations. Since it was found that the pasteurella passes just as readily as *P. pestis* through the carefully shaven skin it was suspected that the integumentum was the receptive organ in the sense of Besredka.[2] A series of experiments on cuti-immunization were carried out. The data are herewith briefly detailed:

Experiment No. 1: Seven strains of rat pasteurella highly pathogenic for guinea pigs were grown in shallow layers (depth 1 cc.) of beefheart-hormone broth (pH 7.0) for 18 days at 28° C. The pooled cultures were filtered through a Berkefeld V Candle under a vacuum of 15 cm. Hg. pressure. The filtrate when tested on solid and liquid mediums was found to be sterile and when seeded with young cultures of the pasteurella failed to encourage further growth. It apparently contained the "anti-virus" of Besredka. However, it was noted that, although no visible growth took place in the tests made for the demonstration of the inhibitive properties of the filtrate, the inoculated bacteria remained viable and vigorously multiplied when the seeded filtrate was diluted with hormone serum broth (20 times its volume). Six guinea pigs received intracutaneously on the abdomen 0.5 cc. of the filtrate. On the following day two animals were dead. Two of the remaining four guinea pigs, which were all sick, died on the second and the last two on the third day after the injection. Postmortem examinations only revealed a marked subcutaneous injection and gelatinous edema immediately adjacent to the site of the inoculation. Smears and cultures proved the absence of bacteria.

Experiment No. 2: A filtrate similar to that used in Experiment No. 1 was prepared. However, the cultures were grown for seven days in 200 cc. of hormone broth held in 250 cc. Erlenmeyer flasks (deep layers). The filtrate was sterile, but when seeded with the pasteurella strains gave a good culture in 24 hours. It contained no "antivirus".

[2] Immunization Locale, Paris, Masson Cie, 1925.

Eight guinea pigs were intracutaneously inoculated with 0.3 cc. of the filtrate. No reddening or swelling was evident on the following day. Twenty-one hours after the injection of the filtrate, the guinea pigs were tested with virulent splenic material containing the pasteurella organism. The tissues were rubbed into the slightly scarified skin or introduced by the pocket method into the area previously injected with the filtrate. The treated animals died as rapidly as the controls infected with the same material and in the same manner. The autopsy findings were typical for hemorrhagic septicemia and were confirmed by smears and cultures.

Experiment No. 3: The filtrate used in this experiment was prepared in the same manner as stated for Experiment No. 1, with the exception that the cultures were incubated for seven days at 28° C. It was sterile, had a pH of 6.4 and contained the "antivirus". Thirty-six guinea pigs were treated by intracutaneous injection of 0.3 cc. of filtrate into the freshly shaven abdominal skin. Twenty-four guinea pigs were injected in the same manner with broth of the identical lot as used in the production of the filtrate. At intervals of 18, 24, 48, 72 hours, 5 and 8 days, four animals treated with filtrate, four injected with broth and four healthy guinea pigs were subjected to immunity tests. The crushed spleen fragments of a guinea pig which had succumbed in 18 hours to the pasteurella infection showing innumerable typical organisms, were applied either on the mucous membrane of the nose or were smeared on the slightly scarified skin or subcutaneously pocketed in the area injected with the filtrate or rubbed on a freshly shaven zone of the back.

The most significant results, which clearly indicate that the guinea pigs intracutaneously injected with broth culture filtrates and subjected to an infection by cutaneous routes are fully protected, are shown in Table I. Since the immunity tests conducted on the 72nd hour, the 5th and 8th days furnished identical results to those presented in the table a detailed presentation is deemed unnecessary. The protection against a fatal cutaneous infection is fully developed on the 18th hour after the treatment and persists for at least three weeks (longest period thus far tested). Not only the vaccinated area but the entire skin is resistant. Furthermore, the immunity is apparently specific since the intracutaneous injection of broth conferred little or no protection. The animals treated with broth succumbed to the test

TABLE I.

Cuti-immunization—Experiment III—Immunization tested 18, 24 and 48 hours after intracutaneous injection of filtrate.

Male guinea pigs weight	Treatment injection on abdomen	Immunity tested with virulent spleen tissue		Results.
200	0.3 cc. filtrate	18 hours after test	Vaccination same area	No symptoms. Normal
195	" " "		Vaccination on back	No symptoms. Normal
210	" " "		Sub. cut. same area	Dead 45 hrs. after test
200	" " "		Smear on nose	No symptoms. Normal
180	" " "	24 hours after test	Vaccination same area	No symptoms. Normal
220	" " "		Vaccination on back	No symptoms. Normal
200	" " "		Sub. cut. same area	Dead 43 hrs. after test
210	" " "		Smear on nose	No symptoms. Normal
240	" " "	48 hours after test	Vaccination same area	No symptoms. Normal
250	" " "		Vaccination on back	No symptoms. Normal
180	" " "		Sub. cut. same area	Dead 44 hrs. after test
185	" " "		Smear on nose	No symptoms. Normal
200	0.3 cc. broth	18 hours after test	Vaccination same area	Dead 69 hrs. after test
250	" " "		Vaccination on back	Dead 72 hrs. after test
200	" " "		Sub. cut. same area	Dead 48 hrs. after test
230	" " "		Smear on nose	No symptoms. Normal
250	" " "	24 hours after test	Vaccination same area	Dead 70 hrs. after test
200	" " "		Vaccination on back	Dead 75 hrs. after test
200	" " "		Sub. cut. same area	Dead 40 hrs. after test
250	" " "		Smear on nose	No symptoms. Normal
200	" " "	48 hours after test	Vaccination same area	Dead 62 hrs. after test
220	" " "		Vaccination on back	Dead 65 hrs. after test
200	" " "		Sub. cut. same area	Dead 42 hrs. after test
250	" " "		Smear on nose	No symptoms. Normal
250	Shave abdomen only	18 hours after test	Vaccination same area	Dead 71 hrs. after test
240	" " "		Vaccination on back	Dead 75 hrs. after test
250	" " "		Sub. cut. same area	Dead 41 hrs. after test
200	" " "		Smear on nose	No symptoms. Normal
210	" " "	24 hours after test	Vaccination same area	Dead 65 hrs. after test
190	" " "		Vaccination on back	Dead 70 hrs. after test
200	" " "		Sub. cut. same area	Dead 39 hrs. after test
230	" " "		Smear on nose	No symptoms. Normal
250	" " "	48 hours after test	Vaccination same area	Dead 69 hrs. after test
210	" " "		Vaccination on back	Dead 71 hrs. after test
250	" " "		Sub. cut. same area	Dead 39 hrs. after test
200	" " "		Smear on nose	No symptoms. Normal

infection after approximately the same lapse of time (69 to 75 hours). The integumentum, even when heavily scarified and virulent material is directly rubbed into the skin capillaries, can fully ward off an infection, while the subcutaneous application of the hemorrhagic septicemia organism produces a fatal disease in the same time as in the non-vaccinated control animals (39 to 48 hours). Twelve guinea pigs intracutaneously vaccinated were bled on the 15th day and the serum tested for agglutinins

and protective substances. No serum antibodies were demonstrated. In view of the publication of Kitt[3] and others, these results were anticipated and other methods must be developed to prove more conclusively the local character of the cuti-immunity. It is reasonable to assume that the subcutaneous test inoculation (pocket method) with a pasteurella organism is too massive to demonstrate the delicate general immunity which might have developed on the 5th and 8th day. Even if such a possibility is admitted one would encounter difficulties in explaining the striking protection of the skin 18 hours after the injection of the filtrate containing a growth inhibiting substance. Experiments are in progress to shed light on the nature of the "antivirus", the duration and mechanism of the cuti-immunity.

Conclusion: Although the skin is not the main receptive organ for the bacteria of the pasteurella group, experiments conducted on guinea pigs have established the possibility of protecting the entire integumentum of the animals inside of 18 hours, but not the subcutaneous tissues, when non-toxic filtrates prepared from seven days old cultures grown in shallow layers, containing a growth inhibiting substance or "antivirus", are inoculated intracutaneously.

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Changes in the alveolar process about the teeth in dogs on experimental diets.

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Studies on inorganic salt metabolism in dogs conducted by one of us (M. R. J.)[1] have shown that skeletal changes and dental defects may be induced in normal puppies on diets which appear

[3] Handbuch d. pathogen. Mikroorgan., 2nd Ed., 1913, vi, 56.

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[1] Jones, Martha R., PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 199.