general symptoms of malaise, slight fever and adenitis.

Of the seven persons vaccinated with the vaccine virus carried through six successive passages on the chick membrane one responded with a typical vaccinoid reaction. The remaining six developed typical vaccinia lesions which passed through the successive stages in the usual manner. General symptoms also were typical of the disease.

The four persons vaccinated with the vaccine virus carried through seventy-five successive passages on the chick membrane all responded with positive takes and developed lesions and symptoms in every respect typical of vaccinia.

Daily observation and comparison of the lesions produced by the regular commercial vaccine and those produced by the vaccine cultivated on the chick membrane showed them to be quite comparable to each other throughout their entire course. The following differences were noted. The lesions developing from the virus cultivated on the chick embryo were slightly milder in their appearance. The different stages of the lesion were delayed about one day, as compared with those appearing from the regular calf vaccine. There was less induration of the surrounding subcutaneous tissues, and the involvement of the adjacent lymph nodes was not quite so extensive. In the pustular stage the lesions from the chick strain of vaccine did not contain as large an amount of pus. The crusts were thinner and more flaky and when separated did not leave so marked a depression as those from the calf strain of virus. On the whole the lesions from the chick strain of vaccine were less painful and caused less discomfort.

The lesions produced by the vaccine virus carried through seventy-five passages on the chick membrane were definitely milder throughout the first eight days of their course, but passed through the successive stages in a typical manner. They then rapidly increased in severity to reach their height on the tenth to the twelfth day when their appearance was quite comparable to the lesions produced by the regular calf strain of virus.

The scars left by all the vaccinations are quite comparable as to size, all averaging about 1 to 1.5 cm in diameter. They are all slightly depressed below the surrounding surface. Those produced by the calf strain of virus are slightly deeper. There is evidently less scar tissue formation in the lesions produced by the chick strain of vaccine, as they feel thinner and are less pitted and wrinkled.

The persons vaccinated with the calf strain of virus and the virus from the sixth passage on the chick were revaccinated nine weeks after the primary vaccinations by the scratch method. A control scarification was made in each case. Those vaccinated with the virus from the seventy-fifth passage were revaccinated in the same manner eight weeks after the primary vaccinations.

The persons vaccinated with the chick strain of virus were revaccinated with a calf strain of vaccine virus prepared by E. Squibb and Sons. Those vaccinated with the calf strain of virus were revaccinated with a strain of chick vaccine from the sixth passage of good potency as tested out on the rabbit.

Except in the case of one person who failed to respond to the primary vaccination typical immune reactions developed in all cases following the revaccination. These were characterized by a slight papule and reddening along the line of scarification which were present after twenty-four hours and reached their maximum intensity between twenty-four and forty-eight hours. By the end of seventy-two hours all reactions had subsided. Several had completely disappeared, while a few were still present as a small papule with very slight reddening along the scarification. Observations on the seventh day showed the lesions had subsided.

Some of the advantages which might be expected from the use of chick embryo vaccine over the calf virus are the ease with which it may be produced at any time fertile hen-eggs are available, absence of bacteria and other contaminating agents, and, if present indications are confirmed, the availability of a fixed strain of virus which does not require mammalian passage to maintain its virulence.

Additional studies are in progress intended further to simplify and improve the technique of chick vaccine production and to test the relative durability of its immunizing effect in man.

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THE EFFECT OF IRON ON THE ESTABLISHMENT OF THE OXIDATION-REDUCTION POTENTIAL OF ALLOXANTIN

ON considering the various instances where heavy metal salts act as catalysts in oxidation-reduction processes, the following observation seems to be of interest in which iron acts as a catalyst for the establishment of an oxidation-reduction potential.

Biilmann and Lund[1] have shown that an acidified solution of alloxantin establishes a definite potential at the blank platinum or gold electrode. The interpretation is based on the well-founded assumption that alloxantin in an aqueous solution is split into dialuric acid and alloxan and that these two substances behave as the components of a reversible oxidation-

[1] E. Biilmann and H. Lund, *Ann. Chim.* (9) 19: 137, 1923.

reduction system. The case of alloxantin is thus analogous to that of quinhydrone. Richardson and Cannan[2] extended the potentiometric study over a wide pH range and performed oxidative potentiometric titration experiments which, in spite of difficulties with respect to the lack of stability of the potentials, led to reasonable results.

The reproduction of Biilmann's experiment is easy, provided one works with rather concentrated solutions of alloxantin (about 0.3 per cent.), best in saturated solution with an excess of solid alloxantin. In this case, of course, oxidative or reductive potentiometric titration experiments can not be performed.

On the occasion of our own potentiometric study of alloxantin it appeared to us striking that the potentials become erratic, inconstant in time and irreproducible, when a somewhat diluted solution of alloxantin is used instead of a saturated one, a fact which has never been mentioned anywhere. A further study showed that on the addition of a small amount of an iron salt the potentials become reproducible and are rapidly established, even in very dilute solutions of alloxantin (1:10,000 and even more dilute). Mere traces of iron are not sufficient to establish the full effect, but well-measurable amounts are requisite. About 0.1 to 1.0 milligram of iron, as sulphate, added to a volume of 25 cc of the solution is necessary. On the other hand, in a concentrated solution of alloxantin, Fe is not requisite. On working with all precautions as Fe-free as possible and adding such powerful Fe-combining reagents as alpha, alpha'-dipyridyl, or alpha, alpha'-phenantrolin the potentials are quickly established in concentrated solutions.

Alloxantin can be reduced in the electrode vessel by hydrogen and colloidal palladium to dialuric acid, and after replacing the hydrogen by nitrogen an oxidative titration experiment with bromine can be performed. The oxidation product is alloxan. The establishment of the potentials is sluggish and erratic. When, however, a small amount of $FeSO_4$ had been added to the mixture, the potentials are immediately established, just as with any reversible dyestuff. The shape of the titration curve is precisely the one of an ordinary organic dyestuff system, with no indication of any intermediary step of oxidation being shown. This intermediary form, known as alloxantin in the crystalline state, does not exist to any appreciable extent in the solution, just as the substance called quinhydrone in its crystalline state does not exist in solution. The titration curve is not influenced at all by the adding even of a great excess of iron.

The effect of the iron is most evident between pH 4 and 6. At lower pH the effect is small and at pH about 1.0, where Biilmann and Lund worked, no appreciable effect of iron can be seen any more. At so low a pH only a concentrated solution of alloxantin establishes a reproducible potential, and this is not influenced at all by iron.

Iron can not be replaced by copper, manganese, cobalt, nickel or organic dyestuffs.

As for the explanation of this effect, it should be recalled that dialuric acid gives a complex compound with iron. This can be shown, however, only in an alkaline solution. Dialuric acid plus $FeCl_3$, plus ammonia, produces a deep violet color. At the pH range 4 to 6, where the effect of iron on the potential is strongest, no evidence of any complex formation can be shown. No color and no precipitate is produced by the iron. If there exists, at pH 4–6, a Fe complex at all, this complex can represent only a very slight fraction of that part of the substance not combined with Fe. Yet, this minute trace of the complex may be considered as the intermediator for the establishment of the potential. Without emphasizing too much this hypothesis, at any rate, this is another case of the catalytic establishment of a potential in a system which is thermodynamically reversible but sluggishly reactive without a catalyst.

It should be recalled[3] also that Fe acts as a catalyst for the oxidation of dialuric acid by molecular oxygen. The pH optimum of this effect is around pH 7, whereas the effect described above has its optimum at pH 4–6.

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