OBSERVATIONS ON EXPERIMENTAL TYPHOID INFECTION OF THE GALL BLADDER IN THE RABBIT.*

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In recent years the question of immunity following antityphoid vaccination, the typhoid carrier problem, and the possibilities of chemotherapy have restimulated investigation of experimental typhoid infections in the lower animals.

A review of the present status of infection in animals below the apes is given by Hailer and Ungermann (1). In the case of the rabbit the original observation dates back to 1891, when Blachstein and Welch (2) noted that, after intravenous inoculation of colon and typhoid bacilli, rabbits may develop a lesion of the gall bladder which may persist for several months. Apparently no further work was done with this lesion at that time, but, in the last few years, infection of the organs of the rabbit and especially of the gall bladder have been used for various purposes by Doerr (3), Koch (4), Chiarolanza (5), Conradi (6), Hailer and Rimpau (7), Hailer and Ungermann (8), Hailer and Wolf (9), Uhlenhuth and Messerschmidt (10), Morgan (11), Johnston (12), Gay and Claypole (13), and Cummins and Cumming (14).

It has been shown that typhoid bacilli can be isolated from the organs for some time after injection, but that the gall bladder lesion is the most persistent source. After intravenous injection, the gall bladder apparently becomes infected by way of the blood stream as well as from the bile. It has also been shown that intravenous inoculation does not regularly result in producing gall bladder lesions and most of the experimenters in chemotherapy have given up this method for the direct inoculation of the gall bladder. The intravenous method of infection, however, more nearly resembles the natural method and has advantages for some purposes.

In this paper the following subjects are considered: (1) patho-

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genicity of a living sensitized vaccine, (2) pathogenicity of first transplant from a living sensitized vaccine, (3) the regular production of lesions, (4) the gall bladder lesion as a test of immunity, (5) the curative effects of vaccines, and (6) the practical bearing of experimental work. Altogether, ninety-seven animals have been used and forty gall bladder lesions have been observed.

PATHOGENICITY OF A LIVING SENSITIZED VACCINE.

The writer's attention was first attracted to the gall bladder lesion as a means of determining whether or not a living sensitized vaccine is pathogenic. As is well known, Metchnikoff and Besredka (15), as a result of their work with experimental typhoid in the chimpanzee, devised a living sensitized vaccine for protection. This vaccine has been criticized as likely to produce carriers, if not the disease itself. Metchnikoff and Besredka's own observations on the possibility of producing carriers in apes and man have been negative. My own experience with rabbits has been the same. Through the kindness of Prof. Besredka I secured some of his vaccine which was living at the time of use, as evidenced by the growth from the vaccine of a typical typhoid organism. The vaccine was given intravenously to nine animals, and subcutaneously to one animal, in doses of two to six cubic centimeters. Two animals were sacrificed on the day following intravenous injection, and cultures made from the bile were sterile. The other animals were examined after one month; cultures from the bile were negative in all cases for typhoid bacilli, and no macroscopic evidence of disease was found. It may, therefore, be concluded that intravenous injection of this vaccine does not produce gall bladder lesions in the rabbit, and by inference that no danger exists of producing carriers in man by subcutaneous injection.

PATHOGENICITY OF THE FIRST TRANSFER OF A LIVING SENSITIZED VACCINE.

While the original vaccine is apparently harmless, the first transplant on agar is pathogenic, as is indicated by the following experiment.

Nov. 18, 1913. A large agar slant was inoculated with vaccine and, after twenty-four hours, the growth was collected in 20 c.c. of salt solution and 6 c.c. were given intravenously to a rabbit weighing 3,000 gm. On Dec. 23, the animal was killed and a well marked lesion of the gall bladder was found and the bile contained a pure culture of typhoid bacilli.

Hence while the vaccine itself is apparently safe, it has possibilities of harm in case it became accidentally deposited on media suitable for multiplication. This is a point of some practical importance in the military service in which ampules may unavoidably be broken in transportation or in camp. According to the results of this experiment, any water or food accidentally contaminated with this vaccine might transmit the disease to non-immunes. It may, therefore, be stated that a living sensitized vaccine is not entirely safe to handle, as is a killed vaccine (16).

ATTEMPTS TO PRODUCE GALL BLADDER LESIONS REGULARLY BY INTRAVENOUS INJECTION.

While the above work was in progress, Gay and Claypole (13) published a paper in which the authors stated that regular infections had been produced by intravenous injection of growths from rabbit blood agar. In order to try the effect of this procedure, the strain from the living vaccine was carried on rabbit blood agar and passed through lesions in five animals, as is seen in table I. The growth on a blood agar slant was suspended in twenty cubic centimeters of salt solution, and two cubic centimeters, or one half the fatal dose, were given intravenously and the animals were examined after one month.

TABLE I.

Attempts to Produce Gall Bladder Lesions with Transfers from Besredka's Vaccine on Blood Agar.

No. of passage.	No. of animals.	Positive.	Negative.
1	1	1	0
2	6	1	5
3	3	1	2
4	1	1	0
5	5	1	4
	16	5 (31%)	11

It will be seen that with this strain cultivation on blood agar had no effect in maintaining pathogenicity, nor did passage through four animals in series. In the fifth transfer only one animal out of five became infected. In this connection it should be noted that the strain Gay and Claypole worked with was recently isolated.

In order to determine the effect of the age of the culture several strains were tried with the following results. One half the fatal dose of an agar or bouillon growth was given intravenously and the animals were examined after one month (table II).

TABLE II.

Attempts to Produce Gall Bladder Lesions with Various Strains Given Intravenously.

Strain.	Age of strain.	No. of animals.	Positive.	Negative.
Clark	1 mo.	8	6	2
Fuguet	1 mo.	2	1	1
Andrews	1 mo.	4	0	4
Clark	3 mos.	4	0	4
Rawlings	14 yrs.	6	0	6
Dorset	15 yrs.	5	1	4
Besredka	?	16	5	11
		45	13 (28.8%)	32

The conclusion to be drawn from these experiments is that freshly isolated strains produce lesions in a higher percentage than old strains, but even freshly isolated strains cannot always be depended on to produce lesions by the intravenous method. This conclusion agrees with that of nearly every investigator and has been emphasized by Uhlenhuth and Messerschmidt, and Hailer and Ungermann. As stated above, these authors have given up the intravenous method for the more certain method of direct inoculation of the gall bladder.

THE GALL BLADDER LESION AS A TEST OF IMMUNITY.

Gay and Claypole also state that the gall bladder lesion can be used as a test of immunity conferred by vaccines. Evidently such a test in an animal would be of great value in many ways, but unfortunately this lesion does not seem to be suitable for such a

purpose. The instantaneous introduction of a large number of bacilli directly into the circulation seems to break down any artificial immunity produced by vaccines. Uhlenhuth and Messerschmidt (10) have been unable to protect animals from infection with the Pfeiffer-Kolle vaccine, although immune bodies were present in large quantities. The infecting dose was given directly into the gall bladder. My experience has been the same with the Army vaccine, using the intravenous route. Although it is impossible to carry on well controlled experiments with the intravenous method when the results are so uncertain, the positive results have some significance.

Ten rabbits were used. Six were vaccinated with 100 million subcutaneously, followed at weekly intervals by two doses of 200 million, intravenously. Four were vaccinated similarly with doses of 500 and 1,000 million. Ten days after the last vaccination, all were given an intravenous injection of about one half the fatal dose of Besredka's organism. After one month, two animals of each set showed definite infections of the gall bladder. The percentage of infections was 40, while the percentage of infections among controls (16) was 31.

Hence no protective effect of vaccination could be demonstrated in the rabbit. As the protective effect of the same vaccine in man is well known, this result simply means that the conditions of the experiment in the rabbit are not comparable with those in natural infections in man.

ATTEMPTS TO CURE THE GALL BLADDER LESION BY VACCINES.

Johnston (12) states that vaccines may have some curative effect on the lesion of the gall bladder. Uhlenhuth and Messerschmidt (10) were unable to obtain such a result, and Cummins and Cumming (14) also state that vaccine treatment did not influence the finding of living bacilli in the liver and gall bladder. I have also been unable to demonstrate any curative effect of vaccines.

Two rabbits were given 1 c.c. of a broth culture of Clark strain intravenously on Apr. 27, 1914. Plating of feces was positive to typhoid bacilli on May 15, 1914. Both animals were then given subcutaneously four doses of vaccine, one dose every five days, running from 10 to 80 million. One week after the last dose the animals were autopsied, and the bile in both cases yielded a pure culture of typhoid bacilli.

Two human carriers who have been studied in this laboratory and reported by Leary (17) were treated over several months with autogenous and stock vaccines and failed to show any marked diminution in the excretion of bacteria. Both cases were operated on and the gall bladders, containing pure cultures of the organism, were removed. Excretion of typhoid bacilli in the stools ceased permanently in nine days. It is, of course, not claimed that removal of the gall bladder will cure all carriers, because we know that the bile passages and intestinal mucosa may be affected in some cases. But our experience with the curative effect of vaccines has not been encouraging either clinically or experimentally.

PRACTICAL BEARING OF EXPERIMENTAL WORK.

In this laboratory our chief interest in experimental infections has naturally centered in their possible relation to immunity following vaccination. As is well known, the present army vaccine was devised in 1908 by Major F. F. Russell of the Medical Corps, on the basis of German and English experience. During the last five years we have manufactured over 1,700,000 cubic centimeters for the Army, Navy, National Guard, and other departments of the Government, and over 250,000 men have been immunized. The results of the use of this product have been fully detailed by Major Russell (18) in a number of contributions to our knowledge of this subject. It is only necessary here to emphasize the fact that the conditions of its use have been those of extensive experimentation. The subjects are exposed to infection in many parts of the world and are under close observation for several years at least. The results have exceeded all expectations, and, on the basis of English and American experience, we can now give a definite answer to some of the problems of immunity in man.

In the first place it is clear that the conclusions reached by Metchnikoff and Besredka from their work on chimpanzees cannot be accepted for man. These authors found that a whole killed vaccine did not protect their animals. But they used tremendous infecting doses,—the contents of a whole Kolle flask. On the other hand, they were able to protect with a living sensitized vaccine. But, as

has already been pointed out, this vaccine is not entirely safe to handle and its use could certainly not be made compulsory. Hence, if we followed these experimental results strictly, we would not be using any vaccine and the splendid results would have been lost. Inasmuch as the final object of all experimental work on this subject is the protection of man, it would be a *reductio ad absurdum* to give up the goal, once reached, because we cannot duplicate the conditions in animals.

In the same way the gall bladder lesion in the rabbit fails as an index of immunity from vaccination in relation to man. For an attack on the typhoid carrier problem this lesion seems ideal, and it may possibly serve in advancing the chemotherapy of typhoid fever. It certainly gives an admirable experimental opportunity for testing methods of isolating typhoid bacilli from the feces. But the claim of Gay and Claypole, that this lesion can be used as a test of immunity from vaccination, seems unfounded, and for the same reason as in the case of the chimpanzees. Human conditions cannot be duplicated and immunization which is known to protect man is not effective under these different conditions.

While the subject was developing, many products were proposed; the whole killed vaccine, autolysates, residues, fractions, etc. When it became evident that a whole killed vaccine had some advantages, other questions arose. What strain should be used? Should it be virulent or avirulent? Should it be fresh or old? Should several strains be used? Should they be sensitized? A great deal of work done with immune bodies has given no convincing answer to these questions. As a result of actual trial we can say that a whole vaccine killed by heat at 53° C. and preserved with 0.25 per cent. tricresol protects human beings from typhoid fever; that an old avirulent strain is effective; that a single strain is effective against typhoid in all parts of the world (19); that doses of 500 and 1,000 million at intervals of seven or ten days are effective; that two doses protect for two years and that three doses protect for a longer period not yet exactly determined; that the vaccine keeps its immunizing properties for at least four months.

Gay and Claypole say that "Empiricism rather than experimentation has largely determined the method employed." On the con-

trary, experimentation has marked every step of the evolution of our present vaccine since the first paper on the subject in 1896, by Pfeiffer and Kolle (20). The only peculiarity has been that the teachings of clinical experience have been given some weight; and that experimental results have been taken for just what they were worth and no more. No claim is made that the Army vaccine is the *summum bonum* in antityphoid vaccines. Improvements may come, but before our results are improved upon, they must be equalled. And they must be equalled, not in a few chimpanzees or rabbits, but in large numbers of human beings under observation for several years.

At present the subjects which give concern are, naturally, the length of immunity and the keeping qualities of the vaccine. Evidence on these points is accumulating from actual experience, and we know of no other safe method of reaching conclusions on these points.

SUMMARY.

1. Besredka's living sensitized vaccine, given intravenously, does not produce a typhoid lesion of the gall bladder in the rabbit.

2. The first transplant of this vaccine is capable of producing this lesion. Hence this vaccine is not entirely safe to handle.

3. Regular infections of the gall bladder have not been produced by carrying a known pathogenic strain on rabbit blood agar, by successive passage through animals, or by the use of freshly isolated strains.

4. No evidence could be demonstrated in the rabbit of the immunity produced in man by vaccination with a whole killed vaccine.

5. Vaccine treatment did not cure the gall bladder lesion.

6. With the present methods of producing infections in the chimpanzee and the rabbit, neither of these animals is suitable for deciding the problems of the immunization of man by vaccines. These problems must be settled, as some of them already have been settled, by actual experience with large numbers of men kept under close observation.

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