and it is as natural as possible. The growth is very profuse, even after eighteen hours, and the emulsion obtained is white.

When counting a vaccine, a culture not more than one day old should be used, since after this time many of the gonococci begin to autolyse, and the count would be too low. After making up standard emulsions of, say, 1,000 and 500 million gonococci per c.cm., the strength of other emulsions can be estimated directly by diluting and matching them with the standard.

I hope to publish later results showing the antigenic power of vaccine prepared on this medium.

A NOTE ON THE VALUE OF BRILLIANT GREEN AS AN ANTISEPTIC.

BY

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THE comparative novelty of the use of brilliant green as an antiseptic for the dressing of wounds is the excuse I submit for the following notes.* Since May, 1916, I have been using and observing the effects of a solution of brilliant green in the treatment of wounds that have passed through my hands at a casualty clearing station. On the whole, I am favourably impressed with the good results obtained from its use.

The brilliant green is dissolved in normal saline solution in the strength of 1 in 1,000. At this strength it can be used as a lotion, and gauze soaked in it can be applied to the wound as a dressing. It is non-irritant to the tissues, and I have applied it to the peritoneum, the meninges, the synovial membranes, and practically all other varieties of tissue without harmful effects. The less vascular tissues are stained green by its use—for instance, the cuticle of the skin, the edges of fascia or aponeurosis, and sometimes bone. But where it has been in contact with the more vascular muscle or subcutaneous tissue no staining occurs. Dead and necrosed portions of muscle are stained green, and this fact is sometimes of use in distinguishing such necrotic tissue.

After being in contact with the tissue, the dye gradually becomes transformed into a leuco-derivative, and the hitherto green-dyed gauze in contact with the wound becomes white to the depth of several layers. Granulation tissue rapidly forms in the wound, and to emphasize this statement I believe that the formation of granulation tissue is more rapid and more "virile" in character under the influence of a dressing of brilliant green than with other antiseptics—for example, eusol.

The most striking results are seen in the cut surfaces of muscle. The muscle rapidly becomes bright red, and the formation of a highly vascular granulation tissue takes place. In thirty-six to forty-eight hours the muscle may be covered with firm, "dry," bright red granulation points, which present none of the shreddy, sodden look of the granulations under a eusol dressing. The surface of the wound is drier, and the pus formation is smaller in amount and thicker in consistency, than in a similar wound dressed with eusol.

At a casualty clearing station it is almost impossible to formulate other than approximate conclusions concerning the merits or demerits of any antiseptic. The obstacles against obtaining any exact judgement are as follows:

1. The cases have been handled before, and other antiseptics have been applied—that is, at the field ambulance.
2. The cases do not stay long enough for any extended observation, before being transferred to the base.
3. Those cases that do stay and on which more lengthy

* The potent antiseptic properties of brilliant green were drawn attention to by Browning and Gilmour (*Journ. Path. and Bact.*, vol. xviii, 1913, p. 144), and this substance was first employed as an antiseptic in the treatment of wounds by Leitch (*BRITISH MEDICAL JOURNAL*, February 12th, 1916); its action under clinical conditions has also been very favourably reported on by Ligat (*BRITISH MEDICAL JOURNAL*, January 20th, 1917), and Hodgson-Jones (*BRITISH MEDICAL JOURNAL*, 1917, i, p. 455) has recorded excellent results in the rapid healing of indolent ulcers under the use of an ointment of 1 to 2 per cent. brilliant green in paraffin. Further investigations by Browning, Gulbrandsen, Kennaway, and Thornton (*BRITISH MEDICAL JOURNAL*, January 20th, 1917) have shown that concentrations of brilliant green which are highly bactericidal for organisms, such as staphylococci, in the presence of serum, do not interfere with the process of phagocytosis; in this respect brilliant green is much superior to mercury perchloride.

observations can be carried out, are of the more severe type, many having mortal wounds.

4. The difficulty of obtaining news of the subsequent history of the case after it has left the casualty clearing station.

However, by a method of comparison of selected cases, as nearly as possible similar in nature and extent of injury, it is possible to estimate the relative merits of two or more antiseptics, as judged by clinical standards. By such means I have tried to compare brilliant green with eusol, and I believe that the green is the better antiseptic of the two.

Latterly I have been using the green in conjunction with "salt" tablets. It has been my experience that the usefulness of the "saline pack" is enhanced by the inclusion of some antiseptic in the dressing. The combination of the "saline pack" with the green solution during the first few days of the wound has, I think, given better results than the use of one or other alone. It can also be used after the method of Carrel— $\frac{1}{2}$ oz. to 1 oz. of a 1 in 1,000 solution being syringed down a tube or series of tubes leading into the depths of the wound. It is not so irritant to the skin edges as the hypochlorite solution.

SUMMARY.

1. Brilliant green is undoubtedly an active and efficient antiseptic.
2. It is non-irritant.
3. It acts well in the presence of serum.
4. It possesses very definite "auxetic" properties.
5. It stains dead tissue green, and in this way may aid the surgeon in determining what to excise.
6. As it is soluble in "saline" it can be used in conjunction with the "salt pack."
7. It can be used after the method of Carrel.

FLIES AND BACILLARY ENTERITIS.

BY

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IN view of the rapidly approaching summer campaign, it seems advisable to draw attention again to the ever-present dangers from flies during the "season." So much has been written on the subject that it might seem almost invidious to direct attention again to our commonest and most familiar domestic pest.

The matter has received thorough treatment by the Local Government Board, and has been taken up to some extent by independent observers. By far the most comprehensive series of observations is that by Graham Smith,¹ extending over a number of years. In his *Flies and Disease* we have the nucleus around which to group further incidental observations and theories.

The limit of the house-fly's potentialities as a disease carrier has not yet been ascertained, and it might with some degree of safety be affirmed that the spread of almost every disease with which is associated an infectious discharge or exudation of one sort or another may be furthered and accelerated by house-flies. Such diseases are common, and not a few of them are well known to reach their maximum incidence at or about the height of the fly season. On the other hand, in no case has the house-fly been definitely proved to be the exclusively specific carrier of any particular disease. In the majority of cases it is merely the most assiduous and most effective. So far as I am aware, the house-fly is not in any case the actual "intermediate host" of any disease-producing organism occurring in man. It appears, however, to be a true intermediate host of at least one parasite of the horse (*Habronema muscae*).

During the course of my tenure of the Ernest Hart Fellowship, I was able to show² that flies had considerable potentialities as disseminators of parasitic worms, and I was led to make investigations on the bacterial flora associated with flies, and in particular on the occurrence of certain organisms pathogenic to man.

In a recent paper (1917) I published the results of a series of experimental investigations on the relation of

flies to the spread of typhoid fever. Some years previously (1911) I published the results of a general investigation of the natural bacterial flora of the house-fly (*Musca domestica*), and noted the occurrence of certain enteritis-producing bacteria such as Morgan's No. 1 bacillus and *B. paratyphosus* B. The flies from which these bacilli were obtained, captured in houses in Battersea, London, were not allowed access subsequently to any possible source of infection, so that there can be no question that they were actually carrying these bacilli when captured.

The first paper was published as a preliminary report before the investigation was completed. During the following summer further observations were made along similar lines, and about 2,000 flies were collected from houses where cases of diarrhoea or dysentery, either in children or adults, were actually present and had recently occurred. The houses were designated by Dr. G. Quin Lennane, M.O.H. Battersea, and in each a recently sterilized balloon trap was left usually for two days, sometimes for only twenty-four hours, and occasionally for three days. During transit each cage was kept as free from contamination as possible. There can be little doubt that while the cages remained in the houses in which they were deposited they were the object of some curiosity and probably considerable handling, but this does not invalidate the results obtained from the later examination of the contents of the cages.

In the laboratory the flies were removed from the cages by sterilized muslin bags. A simpler but somewhat slower method was to place the cage under a large bell jar into which a little chloroform had previously been poured. The flies were sorted into batches of about half a dozen, and each batch was thoroughly washed first in peptone broth, and then in saline, followed by immersion for twenty to thirty minutes in lysol or other disinfectant. They were then washed free from the disinfectant with sterile water, and dried by passing over a flame, and dissected; the entire alimentary canal was removed, and placed in a few drops of sterile peptone broth and thoroughly mashed up. A small loopful of this emulsion was then spread on three successive MacConkey plates (neutral red bile-salt agar), and incubated at 37° C. for about twenty-four hours. From each plate all the non-lactose fermenting colonies were removed, and inoculated separately into peptone broth.

In dealing systematically with the results, the table drawn up by Morgan and Ledingham² was used with slight modifications (suggested by the authors themselves). One of the chief difficulties encountered was the occurrence of slow lactose fermenters. In every case, however, the action on lactose was observed over a period of at least ten days, and such cultures as gave equivocal reactions were subcultured and re-examined. A certain proportion of these slow lactose fermenters which gave reactions somewhat resembling those of the typhoid-dysentery group are excluded from the present results.

On the other hand, there is the difficulty that certain strains of bacteria may be "trained" or "acclimatized," and, though at first unable to ferment lactose, may do so in the course of time, or after repeated subinoculation. Throughout the whole course of these experiments I had the advice of Dr. W. J. Penfold, who was at that time engaged in the special study of bacterial variation. A certain proportion of these equivocal strains were submitted to him for independent examination. In addition to those slow lactose fermenting bacilli a considerable number of true non-lactose fermenters occurred. They were subdivided into two classes, according as their reactions showed affinity with those of the typhoid-dysentery group or not.

Altogether 216 separate forms were isolated. Of these 89 were representatives of Graham Smith's Group C; 40 belonged to Group H; 34 to Group G; 20 to Group A; 13 to each of Groups B and I, and 7 to Group D. Groups E, F, J, K were entirely unrepresented.

These results accord fairly closely with Graham Smith's, who found a large preponderance of organisms of the type of Group C, while those of Groups H, G, and B were relatively much less frequent. As in my experience, Groups E, F, J, and K were entirely unrepresented, while Groups A and D occurred rarely, and Group I not at all.

In analysing the various groups some difficulty was experienced in following Graham Smith's classification,

because in several cases salicin was not available. With this exception the results obtained are closely comparable with those of Graham Smith.

In Group A the organisms found were Nos. 1, 5, and 6. No. 6 was that most frequently found in flies by Smith, while it was also found most frequently in the faeces of diarrhoea cases. Nos. 1 and 5 are the most common types in normal children. In Group B all the varieties belonged to Subgroup B a, and the most commonly occurring members were Nos. 7 and 8, the latter being those most frequently associated with diarrhoea cases.

Group C is one of the largest and most important, and its representatives were by far the commonest in my experience. The most frequent types were C a and C g, 7 and 8; C a 8 and C g 8 were those most commonly found by Graham Smith, and this has been the experience of several other investigators. In my experience C a and C g, 11 and 12 were fairly common. Other types were extremely uncommon. Nos. 2, 3, 4, 5, 6, 10 were not encountered in any of the groups or subgroups. This is in accord with Graham Smith's findings, apart from the fact that he found C g, 5 and 6 fairly commonly.

Group D is exceedingly uncommon, and only a single representative—namely, D d 17—was found. It has been found only once in flies, and exceedingly rarely in children. In Group G, type G a 8 was twice as common as any other. Nos. 6, 9 and 12 were the only others met with. In Group H, which contains a considerable number of relatively infrequent types, half a dozen different varieties were encountered, for the most part referable to the Subgroups a and g. Finally, in I the varieties isolated were—a 10 and c 10.

From this short summary it may be judged that these results, while much less extensive and comprehensive than those of Graham Smith, follow his fairly closely. They serve to show, in the first place, that the non-lactose-fermenting bacterial flora of the fly's intestine is, within a certain range, fairly distinctive, and moreover, that in flies captured in dysentery or diarrhoea areas the bacterial flora is considerably altered in character, the forms found tending to approximate to certain more or less definite types, which appear to be associated with the disease in children—or rather, perhaps, with the conditions which accompany or give rise to the disease.

Of the bacteria which have been specifically identified with the disease, none is better known, probably, than Morgan's No. 1 bacillus (G a). Whether this be the actual causal organism or not, there can be little question that it is a fairly common inhabitant of the intestine of flies, particularly during the summer months.

The occurrence of *B. paratyphosus* B in flies in the natural state is of considerable importance. It may be, however, that, as appears to be the case with *B. typhosus*, it is only a fortuitous inhabitant of the fly, and that in ordinary circumstances it is more or less rapidly eliminated.

The chief point to be determined in regard to the relation of flies to enteric diseases appears to be whether the pathogenic bacteria ingested by flies are eventually excreted without appreciable loss of virulence. It has been shown that in the case of *B. typhosus* the bacilli may be excreted by the fly for a short time, and that such bacilli may retain their serological properties and characters to such degree as to render them indistinguishable from virulent organisms of the same kind. It is, however, hardly to be expected that the short sojourn of, it may be, forty-eight or twenty-four hours or less in the fly's intestine can have much effect upon the pathogenic properties of the bacteria. Were it otherwise, one might at least expect some change, even though slight, in their biological properties. This matter, nevertheless, appears to be worth some further investigation.

CONCLUSIONS.

It has been fairly conclusively proved:

That under experimental conditions flies can readily carry and disseminate certain pathogenic organisms.

That these organisms are not infrequently met with in flies under natural conditions, particularly in association with outbreaks of infectious diseases.

That organisms producing bacillary enteritis are to be met with not infrequently in flies under natural conditions.

That a considerable number of organisms resembling

enteritis bacilli occur frequently in flies in the natural state, and that the utmost care is necessary in discriminating between these and the true enteritis-producing organisms.

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The Croonian Lectures

ON

ADAPTATION AND DISEASE.

DELIVERED AT THE ROYAL COLLEGE OF PHYSICIANS,
JUNE 21ST, 1917.

By J. G. ADAMI, M.D., F.R.S.,
LIEUT.-COLONEL C.A.M.C.

LECTURE III.

ADAPTATION TO DISEASE-PRODUCING AGENCIES IN THE
HIGHER ANIMALS.

(Abstract.)

FROM the data brought forward thus far the following conclusions can reasonably be drawn:

1. The evidence is abundant and conclusive that bacteria are capable of being modified by alterations of environment of certain orders.
2. The modifications conform with Herbert Spencer's "direct adaptations."
3. It can be shown that not some, but all, the microbes subjected to particular orders of alteration of environment exhibit the particular modifications: the hypothesis of "chance variation" in one direction with survival of the fittest is incapable of explaining the phenomena.

After calling attention to two examples of direct adaptation in protozoa higher than the bacteria, namely, the conversion of negative into positive chemotaxis in the myxomycete, *Fuligo*, and Musgrave and Clegg's observations upon the accustomance of entamoebae from the intestine to new bacterial and tissue foodstuffs, it was pointed out that the overwhelming studies of the last quarter of a century upon immunity afford the deepest insight into the processes involved in direct adaptation in the higher animals. There is a vast literature upon the subject. Some of the most notable achievements in science of our generation have been in this particular province. The names of Pasteur, Koch, Ehrlich, Metchnikoff, Almroth Wright, are household words. Every intelligent being knows something about tuberculin, diphtheria antitoxin, phagocytosis, and typhoid inoculation. Yet no single general biologist has dealt seriously with the significance of these discoveries in relationship to adaptation and evolution.

Typical examples were afforded demonstrating that immunization of the individual is direct adaptation. The cases of the phytotoxins, abrin and ricin, were considered in the first place. Both are intensely poisonous in minute doses. Against both the animals of the laboratory can be immunized so that they can withstand 500 or 5,000 times the lethal dose; the blood serum of the immunized animals contains bodies which neutralize the toxin. Evidence was given showing that the cells of the body of the immunized animals elaborate and discharge the antitoxin in great excess over the amount of toxin originally introduced; that they continue to elaborate this for weeks and months after the introduction of the toxin. We deal with the acquirement of a new property which cannot possibly be regarded as the calling into activity of a property previously possessed; the acquirement is something positive; there can be no alternative hypothesis of loss of inhibiting factors; no possibility of ascribing the new property to the persistence of a chance variation. Antiricin can be

produced in rabbit or mouse with absolute certainty, and there is here no alternative explanation of the survival of the fittest.

The parallel examples of the production of diphtheria and tetanus antitoxins were then taken up, and next it was shown that the cells which absorb and fix the toxins produce the antitoxins. Following this, the more widespread bacteriolytic immunity developed against members of the typhoid-coli group, cholera spirilla, micrococci, etc., was studied and shown to be an acquired power of digestion of unfamiliar proteins, especial reference being made to Pfeiffer's reaction. Nor is the acquirement of necessity temporary in the individual. It may last for months and even years. Reference was made to the agglutinating powers of the blood of those recovered from typhoid fever months and sometimes years after complete convalescence.

This continued production exemplifies what Weigert and Fraser Harris had referred to as the "law of inertia." In the order of events here discussed the lecturer held that the activity once started continued too long to be comparable with physical inertia or momentum; there seemed to be the setting in motion of a serial or cyclic series of inter-cellular reactions and counter-actions, the one action starting the other. He preferred, therefore, to employ for the present the non-committal term of "the law of habit."

Numerous examples were given of the working of this law of habit in association with morbid states of various orders in man, of the survival of symptoms long after the agent causing the symptoms had disappeared, particular reference being made to the tics, hysterical and neurotic manifestations; the continued production of antitoxins and bacteriolysins was of the same order; he held that this same law explained the metaplasias and neoplasias.

As to the inheritance of these acquired conditions in the higher animals, he pointed out the restrictions induced by conjugation and amphimixis, the difficulties introduced in mammals by the intrauterine existence of the fetus, and in man by social customs. Two conditions have to be taken into account—namely, those of indirect and direct (or identical) inheritance.

Regarding indirect inheritance, it has to be observed that the germ cells are not so sacrosanct as to be insusceptible to influences affecting the body at large. The germ cells have to absorb foods and grow. If the lymph contain soluble substances the germ cells are not precluded from absorbing and being affected by them. And that this does occur is well shown by the studies upon the effects of paternal intoxication with lead, mercury nitrate, products of the tubercle bacillus, abrin, etc., the mother not being exposed to the poisons. In all these cases reduction in the number of pregnancies is noted, with increase in the number of stillbirths, liability to death within a few days after birth, and the production of an impoverished and highly susceptible offspring, but a small proportion of the progeny reaching maturity. Lustig and Watson (with abrin), Carrière (with tuberculin) note, in addition, an increased susceptibility on the part of the offspring towards the specific poison.

Of all these observations the most clear and decisive are those of Professor Stockard, of Cornell University. He subjected male guinea-pigs daily for a period to the fumes of alcohol, and mating them with normal females obtained but five living litters from twenty-four matings, and of the twelve offspring, seven died in convulsions soon after birth; the survivors, when two months old, were half the size of control guinea-pigs of the same age. When now the nervous and undersized members of this second generation were mated, even when unrelated and never themselves exposed to alcohol, their offspring tended to be still more degenerate, and to show gross deformities. Mating together unrelated members of this third generation, in the few examples observed, gave even more unfavourable results. In other words, two alcoholized great-grandfathers influenced the progeny down to the fourth generation.

We have here the most precise evidence of the inheritance of acquired defects, evidence that fits in wholly with our routine medical experience of the danger of marriage in families which exhibit like nervous or other defects. With these experiments before us it is absurd to postulate that defects of these orders are atavistic, due to properties