THE CONDITIONS AND CHARACTERS OF THE IMMUNITY PRODUCED IN THE GUINEA PIG BY INSTILLATION OF HORSE SERUM INTO THE NOSE.

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In a recent communication[1] we showed in detail that when a few drops of horse serum are instilled, on two or more occasions, into the nostrils of a guinea pig the animal may become affected in radically different ways. An intravenous injection of the antigen given 16 days after the last instillation may lead to more or less profound shock or speedy anaphylactic death. But in a certain proportion of cases the toxic injection produces no obvious reaction. It would appear that the animal had not absorbed the serum introduced into the nose were it not that a second toxic injection, given 24 days after the first, may also be withstood. Therefore, we must conclude that the guinea pig was made primarily refractory, not sensitive to the dose of horse serum introduced into the vein. It seemed to us important to determine the experimental conditions according to which a series of nasal instillations of serum would, on the one hand, render the guinea pig hypersusceptible, or, on the other, insusceptible to a toxic dose of the serum. Our work last year was based on the hypothesis that the biological results of the nasal treatment depended on the time intervals between successive instillations. But, although it became plain that the rhythm of dosages by the nose was not a matter of indifference, we were unable to explain by it the variable effects of the toxic injection.

A more favorable issue has attended the present series of experiments which was suggested through an occurrence which implied a definite relation between the amount of antigen and its qualitative

[1] Sewall, H., and Powell, C., *Arch. Int. Med.*, 1915, xvi, 605.

effects. Immediately, therefore, protocols were prepared for the investigation of the influence of the quantity of serum introduced into the nose upon the specific reactivity of the guinea pig.

The Qualitative Effects of Serum Introduced into the Nose Depend upon the Quantity Instilled.

Our experiments were performed upon young animals, averaging at the outset about 300 gm. in weight. The undiluted Cutter's horse serum was dropped into the nostrils of the animals in the manner previously described.[1] Each drop represented approximately 0.02 cc. The first intravenous or toxic injection, of 0.38 cc., was uniformly given 16 days after the last instillation; the second intravenous injection usually followed the first in 14 days.

Some excerpts from our general results are collected in Table I. The animals in each group were simultaneously carried through

TABLE I.

The Influence of the Quantity of Serum Instilled on the Reaction to Toxic Injections.

Group of guinea pig.	No. in each group.	Amount of serum used in each instillation.	Total no. of instillations.	Intervals between instillations.	No. surviving 1st intravenous injection of 0.38 cc. serum.	No. surviving 2nd intravenous injection of 0.38 cc. serum.
		cc.		*days*		
I	3	0.2	4*	1	0	
II	4	0.2	6†	2	0	
III	2	0.2	4	2	1‡	1
IV	6	0.1	6	2	4	2
V	2	0.04	4	2	2	2
VI	6	0.04	6	2	6	3
VII	6	0.04	12	1	5	3
VIII	6	0.02	12	1	6	4

* A fifth instillation of 0.2 cc. was given 14 days after the fourth.

† A seventh instillation was given 14 days after the sixth and the toxic injection delayed correspondingly.

‡ The amount of serum in the first intravenous injection was reduced to 0.25 cc.

identical procedures. It will be observed that four separate instillations of serum are sufficient to provoke a maximal biologic response. Of nine guinea pigs receiving instillations of 0.2 cc. in quantity (Groups I, II, and III), only one survived the first toxic injection and in this case the amount of serum given by the vein was reduced to 0.25 cc. From the reaction manifested by the animal it is probable that it also would have succumbed to the usual injection of 0.38 cc.

Of twenty guinea pigs receiving instillations of 0.04 cc. or less only one succumbed to the first intravenous injection of 0.38 cc. of horse serum. Twelve of these twenty animals withstood a second intravenous injection of 0.38 cc. of serum 14 days or more after the first. There can be no doubt, therefore, that three-fifths of the animals were strongly immunized by the preliminary instillations of serum. With regard to the seven guinea pigs which succumbed to the second toxic injection the question arises: Did they completely fail to absorb the serum instilled and thus become sensitized by the first toxic injection in the ordinary way, or was the protection conferred by the instillations insufficient in degree to balance the shock of the large second injection? The following considerations led us to adopt the latter explanation.

Many observations have impressed us with the conclusion that the fatality attending the second toxic injection is roughly proportionate to the degree of anaphylactic reaction manifested with the first.

In reviewing our notes we find that of 81 guinea pigs in which record was made of the degree of reaction manifested to intravenous injections following a preliminary course of nasal instillations, in eighteen cases little or no definite shock was caused by the first intravenous injection; of these only three animals succumbed to the second injection of 0.38 cc. of serum, a mortality of less than 17 per cent. Of thirty-nine animals surviving the first intravenous injection after greater or less shock no less than twelve succumbed to the second injection, a mortality of more than 30 per cent.

Another reason for believing that some degree of absorption attends the application of minute quantities of foreign serum to the mucous membrane of the nose consists in the fact that invariably a secretion of saliva follows within a few seconds the fall of a single drop of serum into the nostril; when the lower lip of the animal is

depressed saliva is seen to accumulate about the incisor teeth, provided it has not been swallowed as formed. This has been assumed by us to indicate absorption.

If the death of our animals with the second toxic injection is due, as we suppose, not to inertness of the nasal instillations of serum but to the insufficiency of their immunizing power, it should be easy to demonstrate a weak protective power in the instillations by reducing the amount of the second toxic injection. This we have done to some extent and have found that when the quantity of serum in the second toxic injection is reduced one-half the animals usually survive. There still remains, however, a certain proportion of animals in which the grade of immunity is still much too low to resist this amount of antigen. It is an impression gained from many experiences, but not especially investigated, that the mortality from the second intravenous injection is higher, other things remaining the same, when the second injection follows the first after an interval of 14 days rather than 24 days, the interval formerly employed by us.

The Amount of Serum Absorbed and Its Biologic Effect Depend upon the Method of Instillation.

In all our experiments the complex living mechanisms responded exactly, as depicted in Table I, to the crude method we employed to introduce the serum into the body. In our confirmatory experiments a series of irregularities fortunately developed, which, when investigated, led to a better understanding of the conditions of biologic response. Thus, in a group of six guinea pigs instilled six times on alternate days with 0.2 cc. of serum, it was expected that all would succumb to the toxic injection of 0.38 cc. given 16 days after the last instillation. On the contrary, three of the animals survived, one after very severe shock and two with little or none. The second intravenous injection was reduced to 0.19 cc., and given 30 days later. The animal which had been previously shocked died, the other two easily survived.

Again six guinea pigs were instilled as above, but the quantity of serum used at each instillation was reduced to 0.04 cc. It was expected that all would survive the first intravenous injection of 0.38

cc. of serum. On the contrary, four animals succumbed to it. It was then realized that the method of instilling the serum had been radically different in the two sets of experiments. In the first case the procedure of instillation had been hurried; in the second it was prolonged and attended by obvious vital reactions, such as abundant salivary secretion. It was necessary to determine whether our results could be definitely modified by varying the area of contact between the serum and mucous membrane.

When the head of the animal receiving the instillation is held with its long axis in a vertical plane the serum is probably confined to the respiratory canal and does not reach the turbinate mucous membrane. But if, while the guinea pig is held in the supine position, the head is well extended, the opportunity is given for the serum to gravitate through the complex turbinate convolutions, and this to an extent dependent on the time during which the posture is maintained.

Accordingly, two groups of four guinea pigs each were prepared to test this reasoning. All the animals received five instillations of 0.1 cc. of serum on alternate days. But in the first group the heads of the animals were held vertically and the serum was dropped quickly, from 15 to 30 seconds being consumed in administering the five drops of each dose. In the second group of animals the heads were held well extended and from 3 to 5 minutes were occupied in each instillation. The first toxic injection of 0.38 cc. of serum was given after the usual interval. All four guinea pigs of the first group easily survived; three of the second group died and the remaining one was strongly shocked. We therefore conclude that the biologic effect of a given dose of serum depends chiefly upon the extent of its contact with the mucous membrane of the turbinate apparatus.

It is probable that several other factors, including quality and temperature of the horse serum, take part in determining the coefficient of absorption.

As will be shown in the following section, great individual differences may distinguish animals in their reaction to the same treatment. The susceptibility to anaphylaxis of different families of guinea pigs is noteworthy. Thus, twelve belonging to a wholly different stock from that from which most of our animals were obtained

during the present year proved peculiarly sensitive to serum treatment. Three of them instilled four times with 0.2 cc. of serum on alternate days succumbed to the usual toxic injection, as was to have been expected. The other nine animals received like treatment but with only 0.04 cc. of serum in each instillation. The first intravenous injection of 0.38 cc. of serum killed one and shocked the remainder to a considerably greater degree than usual under these conditions.

Gradations of Sensibility Induced by Serum Instillations.

The reaction to an antigen exhibited by an immunized animal is expressed by a ratio one factor of which is the vital resistance of the host and the other the amount and virulence of the antigen.

In terms of physiology, the plane of immunity is determined by the threshold of irritability to the antigen. This has recently been well brought out by Webb[2] who has successfully inoculated guinea pigs with many thousands of tubercle bacilli of which the minimal lethal dose was 125; but he always failed to establish immunity against recent cultures of which the m.l.d. was only ten bacilli. When a guinea pig is submitted to a series of sensitizing instillations of horse serum it sometimes happens, in possibly 5 per cent of the cases, that with the terminal treatment 8 or 10 days following the first, the animal develops a pronounced attack of asthma with loud, moist bronchial râles.

This is a sign of intense sensitization; we have found the hypersensitiveness to persist at least 60 days and such animals invariably succumb to the first toxic injection as used by us. After three or four sensitizing instillations, another of the same kind repeated after the lapse of 2 weeks produces a greater or less respiratory disturbance in the majority of cases. Such animals succumb to the toxic injection, as do also some which have shown no asthmatic symptoms. Several of our animals which after courses of nasal instillation had been strongly immunized by intravenous injections of serum, when given a nasal instillation of serum after the lapse of some months, showed no reaction whatever. Formerly we were of the opinion that the expression of local sensitization as manifested by asthma required a defi-

[2] Webb, G. B., *J. Lab. and Clin. Med.*, 1916, i, 414.

nite incubation period of 8 or 10 days between the first nasal instillation and that which could produce asthmatic symptoms. Recently, however, we have found that in guinea pigs born of treated mothers and highly sensitized by the subcutaneous injection of 0.1 cc. of horse serum more than 3 months before, well marked asthma could be aroused by the first instillation of serum into the nose. The subject achieves peculiar practical importance in view of the analogous clinical asthma in human beings. Our demonstration of the variability of symptoms of local sensitization of the respiratory apparatus on a constant background of general sensitization makes it questionable as to how reliable tests performed on other peripheral mechanisms, such as the skin, may be for indicating the immunological state of the body as a whole.

The Earlier Instillations of a Series Determine the Biologic Effect of the Whole.

Such results as those depicted in Table I have led us to differentiate our dosages of serum into those which are protective and those which are sensitizing. Previous observations had led to the impression that the two biologic states could be developed one from the other by appropriate intranasal treatment. The following experiments indicate that such is not the case.

To four young guinea pigs were given by the nose six instillations of 0.04 cc. of serum on alternate days. This had been found to be a protective or immunizing treatment. In two of the animals the instillations were continued for four doses but the amount of the serum instilled was increased to 0.2 cc. The remaining two guinea pigs were allowed to rest 16 days and then were likewise given four nasal instillations of 0.2 cc. of serum. Both groups were given intravenous injections of serum 16 days after the last instillations and all the animals survived, with slight symptoms in some cases. A second intravenous injection of 0.38 cc. was likewise survived by all the animals except one of the first group.

In our previous work[1] we had found that as few as two instillations of serum were capable of rendering animals either fatally sensitive to a toxic injection or of inducing an immunity through which

they were able to resist a series of intravenous doses. In Table I several instances are given in which the biologic attitude is determined by a series of four instillations. This seems to us to be a matter of great practical importance and it will be expanded in the final discussion.

Inertness of Serum Administered by the Mouth.

An inconclusive but suggestive experiment was performed upon two guinea pigs by administering four instillations of serum orally. The serum, to the amount of 0.2 cc., was dropped under the tongue on alternate days. The animals showed no response whatever to the first toxic injection and died with the second. The conclusion is that the serum had not been absorbed from the mouth or had been so greatly diluted with saliva as to be ineffective, and that the animals became sensitized by the first injection.

The Biologic Effects of Serum Instilled into the Nose Are of Temporary Duration.

It is generally admitted that guinea pigs sensitized by subcutaneous or other parenteral avenues of injection retain their sensitiveness throughout life. In a former course of experiments we demonstrated fatal anaphylaxis in two guinea pigs that had received the last of a series of nasal instillations of serum 40 days before. But recent observations have indicated to us that immunizing phenomena established through the mucous membrane of the nose are of temporary duration. Two groups were treated, one with a series of six protective instillations of 0.04 cc. and the other with sensitizing doses of 0.2 cc. of serum. 51 days later intravenous injections of 0.38 cc. of serum were given to one animal of the former and two of the latter group. Slight disturbance was manifested by the last two animals, and all three animals died with the second toxic injection administered 15 days later.

Two guinea pigs were given a series of six protective instillations of 0.04 cc. of serum on alternate days. 93 days later each animal received an intravenous injection of 0.38 cc. of serum without response. 14 days later one was given a second intravenous injection

of 0.38 cc. and died. The other animal was given only 0.25 cc. and lived after a moderate reaction.

Two guinea pigs received six sensitizing instillations of 0.2 cc. on alternate days. The first intravenous injection of 0.38 cc. of serum given 51 days later was borne with slight response; a similar injection repeated in 15 days killed both animals.

Three guinea pigs were prepared by six instillations of 0.2 cc. of horse serum. One of these developed an attack of asthma with the last instillation. 60 days later a toxic injection of 0.38 cc. of serum killed the asthmatic guinea pig but produced slight response in the other two. 15 days later one of the remaining animals was given by the vein 0.19 cc. and succumbed after some resistance, the other was given 0.12 cc. of serum and survived with slight response.

Our conclusion is that the immunizing phenomena set up by unsupported nasal instillations of serum gradually disappear within about 3 months, the hypersensitiveness from large instillations first changing to a measure of resistance.

As will be seen later, when relatively large injections of serum are given by the vein to animals within the period of protection afforded by appropriate nasal instillations, the immunity produced is apparently permanent and intensified with time.

In Guinea Pigs Immunized after Preliminary Nasal Instillations the Immunity Is Strengthened with Lapse of Time.

In the paper referred to above we quoted the conclusions of Gay and Southard,[3] to the effect that artificial immunization produced by the injection of serum is but a condition of temporary refractoriness, full sensitiveness returning to the animals if they are kept long enough. How long this period must be is not stated, but our results with guinea pigs immunized through the nose and later treated with a series of intravenous injections of serum led to radically different conclusions. We showed reason to believe that when guinea pigs so handled were brought to resist easily the intravenous injection of a certain amount of serum, say 0.38 cc., with the lapse of time, at least up to 101 days, there was such an increase of refractoriness that

[3] Gay, F. P., and Southard, E. E., *J. Med. Research*, 1908, xviii, 407.

more than 1.0 cc. of serum given intravenously could be equally well tolerated.

We have confirmed our previous findings in the present investigation and at the same time studied a small number of controls immunized through the peritoneal cavity.

Table II gives the histories of fifteen guinea pigs, the last three of which are borrowed from the records of former work. Each of these animals had been prepared by a course of nasal instillations of horse serum, followed at stated intervals by intravenous injections of the same. In each column is recorded the number of days elapsing between the successive injections, and the whole time intervening between the last nasal instillation and the last intravenous injection is represented by the sum of these intervals, *e.g.*, in the case of Guinea Pig 4, this is 102 days. The amount of serum used at each injection is also noted, and the signs L_0 to L+++ +indicate varying degrees of anaphylactic reaction, while D shows that death followed the dose given. Deferring consideration of Guinea Pigs 10, 11, and 12, we see that after a certain degree of resistance had been established, the tolerance of the animals against serum injected into the vein apparently progressively increased with the lapse of time without treatment. In Animals 1 to 3, inclusive, the nasal instillations of 0.2 cc. of serum plainly caused sensitization so that repeated small intravenous injections were necessary to establish a fair degree of tolerance. This is especially noticeable in Guinea Pig 3, which was one of a group of four animals similarly prepared, the others having succumbed early to small injections. Even so it is seen that an extraordinary increase of resistance develops with time after the fourth intravenous injection. It is obvious that there is some metabolic strain towards an equilibrium of increased resistance against disturbance by the antigen. A comparison of these cases with those of Table III, in which the preparation was by relatively large intraperitoneal injections of serum, shows that in the latter animals what we call the metabolic strain is towards an equilibrium of hypersensitiveness.

Guinea Pigs 4 to 15 of Table II were prepared by small nasal instillations such as we have found to confer primary protection. It is seen that the rule is that such a course of instillations reinforced by two intravenous injections is sufficient to establish a ten-

TABLE II.

The Influence of Time on the Development of Immunity in Guinea Pigs Prepared by Nasal Instillation and Tested by the Intravenous Injection of Horse Serum.

No. of guinea pig.	Preliminary treatment.	First intravenous injection. 1. Amount of serum. 2. No. of days since last instillation. 3. Result.	1. Amount of serum. 2. No. of days since last injection. 3. Result.				
			Second intravenous injection.	Third intravenous injection.	Fourth intravenous injection.	Fifth intravenous injection.	Sixth intravenous injection.
1	Six instillations of 0.2 cc. serum on alternate days.	0.06 cc. 13 L++	0.06 cc. 23 L_0	0.19 cc. 16 L+	0.19 cc. 19 L	0.75 cc. 92 L++	0.75 cc. 114 L+
2	Same as No. 1.	0.06 cc. 13 L++	0.13 cc. 23 L+	0.25 cc. 16 L++	0.25 cc. 19 L+	0.75 cc. 92 L++	
3	Five instillations of 0.2 cc. at intervals of 1 to 4 days. (Very sensitive.)	0.02 cc. 15 L++	0.05 cc. 21 L++	0.13 cc. 16 L+++	0.13 cc. 19 L+	0.5 cc. 96 L++	0.75 cc. 125 L+
4	Six instillations of 0.1 cc. on alternate days.	0.38 cc. 16 L	0.38 cc. 16 L++++	0.75 cc. 70 L			
5	Six instillations of 0.04 cc. on alternate days.	0.38 cc. 16 L_0	0.38 cc. 16 L+	0.88 cc. 69 L+			
6	Same as No. 5.	0.38 cc. 16 L+	0.38 cc. 16 L++	0.81 cc. 69 D			
7	Same as No. 5.	0.36 cc. 16 L_0	0.38 cc. 16 L++	0.75 cc. 67 L++			
8	Six instillations of 0.04 cc. followed by four of 0.2 cc. on alternate days.	0.31 cc. 16 L+	0.38 cc. 14 D				

TABLE II.—*Concluded.*

No. of guinea pig.	Preliminary treatment.	First intravenous injection. 1. Amount of serum. 2. No. of days since last instillation. 3. Result.	1. Amount of serum. 2. No. of days since last injection. 3. Result. Second intravenous injection.	Third intravenous injection.	Fourth intravenous injection.	Fifth intravenous injection.	Sixth intravenous injection.
9	Same as No. 8.	0.38 cc. 16 L	0.5 cc. 68 L+	0.75 cc. 122 L+++			
10	Twelve instillations of 0.02 cc. daily.	0.31 cc. 17 L	0.38 cc. 16 L++	0.75 cc. 64 D			
11	Same as No. 10.	0.38 cc. 17 L	0.38 cc. 16 L++	0.75 cc. 64 D			
12	Same as No. 10.	0.31 cc. 17 L_0	0.38 cc. 16 L++++	0.75 cc. 111 D			
13	Six instillations ± 0.15 cc. at intervals of 14 days.	0.38 cc. 16 L_0	0.38 cc. 24 L++	0.38 cc. 75 L_0	1.1 cc. 36 L+++		
14	Same as No. 13.	0.38 cc. 16 L++	0.38 cc. 24 L+++	0.38 cc. 75 L_0	1.13 cc. 101 L		
15	Same as No. 13.	0.38 cc. 16 L_0	0.38 cc. 24 L	0.38 cc. 75 L_0	1.13 cc. 101 L		

dency, elaborated with time, towards a greatly strengthened degree of immunity. Particular attention is called to a comparison of the histories of Guinea Pigs 8 and 9. Both had received essentially the same preparation but Guinea Pig 8 succumbed to the second intravenous injection of 0.38 cc. of serum 14 days after the first. Guinea Pig 9 was kept until 68 days after the first injection and then easily withstood 0.5 cc. of serum. Still more to the point is comparison of the records of Guinea Pigs 13 to 15. Each withstood the third in-

travenous injection of 0.38 cc. of serum without obvious reaction. 36 days later Guinea Pig 13 was given an intravenous injection increased to 1.1 cc. and nearly died. The remaining two animals did not receive their fourth intravenous injection until 101 days after the third; each then withstood with hardly perceptible shock the large amount of 1.13 cc. of serum by the vein. It will be noted that the reactions of Guinea Pigs, 10, 11, and 12 are wholly different from those described above. These animals received a preparatory nasal treatment of twelve instillations repeated at intervals of 24 hours instead of on alternate days. Several diverse experiences have led us to conclude that qualitative differences exist between the immunological response elicited in animals according as they receive successive instillations of serum within one or two or more days.

TABLE III.

The Influence of Time on the Development of Immunity in Guinea Pigs Prepared by Intraperitoneal Injection and Tested by Intravenous Injection of Horse Serum.

No. of guinea pig.	Preparation.	First intravenous injection. 1. Amount of serum. 2. No. of days since last intraperitoneal injection. 3. Result.	1. Amount of serum. 2. No. of days since last intravenous injection. 3. Result.					
			Second intravenous injection.	Third intravenous injection.	Fourth intravenous injection.	Fifth intravenous injection.	Sixth intravenous injection.	Seventh intravenous injection.
1	All animals were given six intraperitoneal injections of serum within a period of 10 days.	0.19 cc. 9 L++	0.2 cc. 22 L++++	0.31 cc. 16 L++	0.31 cc. 19 L+	0.31 cc. 78 L+	0.63 cc. 45 L++	0.63 cc. 116 L+++
2		0.31 cc. 9 L+	0.32 cc. 22 L+	0.38 cc. 16 L	0.38 cc. 19 L+	0.38 cc. 78 L	0.75 cc. 45 L+	0.75 cc. 116 L+++
3		0.2 cc. 32 L++	0.25 cc. 24 L++++	0.25 cc. 12 L	0.75 cc. 94 D			
4		0.2 cc. 32 L+	0.26 cc. 18 L+	0.25 cc. 18 L+	0.38 cc. 94 D			
5		0.23 cc. 32 L++	0.33 cc. 18 L	0.33 cc. 18 L++	0.38 cc. 95 D			

Table III represents the histories of five guinea pigs which received courses of intravenous injections of horse serum after a preparatory period in which six intraperitoneal injections of 0.5 cc. of serum were given within 10 days. Comparing the animals with Nos. 1, 2, and 3 of Table II we see that they were capable of tolerating a much larger initial intravenous injection than the latter, but in Table III the tolerance of the subjects to increasing doses of the antigen is shown to have increased more slowly than in Table II. But the fundamental difference is that the guinea pigs of Table III, after having been made relatively immune to a certain intravenous dosage of serum, when kept for 3 months or more and then reinjected, manifested an increase of susceptibility, whereas in Table II the contrary is the case under similar conditions.

It should be expected that Guinea Pigs 1 and 2 of Table III would have been thoroughly immunized by the long succession of intravenous injections. Nevertheless we find that when a seventh injection was given following a resting period of 116 days after the sixth the animals were much more profoundly shocked by the dose which had previously been fairly well tolerated. They would undoubtedly have succumbed to any such increase of dosage as was employed with impunity on the subjects of Table II.

General Hypersensitiveness Is Not Abolished by Intranasal Treatment.

Since it has been shown that immunity to toxic injections could be established by instillations into the nose of definite, small amounts of horse serum, that is, that intranasal treatment might be used to produce with certainty prophylaxis against anaphylaxis, it was important to discover whether the cure of a condition of serum hypersensitiveness might be effected in a similar manner. Several experiments were undertaken with this end in view in our work already reported. The results were uniformly negative; no animal which had been sensitized by subcutaneous injection of horse serum and was subsequently treated by nasal instillations of serum survived an intravenous injection of 0.25 cc. afterwards. Since learning the fundamental biologic importance of the amount of serum used in the instillations

it was thought well to repeat these experiments. Our results have thus far been uniformly negative, but in view of the practical importance of the subject the investigation is still being pursued.

DISCUSSION.

The results which have been described strengthen the hypothesis on which the work was founded; namely, that the mucous membrane of the nose is an avenue to the mechanism of immunity which offers peculiar advantages over parenteral routes. Nothing less should be expected if, as seems probable, nasal absorption is a normal stage in the development of natural immunity. We have shown that a guinea pig which has been treated by a series of four nasal instillations of 0.04 cc. of horse serum on alternate days may withstand 16 days later the relatively enormous toxic injection of 0.38 cc. with hardly perceptible reaction. It would appear that the instilled serum had not been absorbed by the nose were it not that a second toxic injection given 15 days after the first may likewise be easily tolerated.

Reasons have been given for believing that every application of as much as 0.02 cc. of horse serum to the mucous membrane of the nose of the guinea pig is attended with sufficient absorption to produce systemic effect. The biologic result of this absorption is qualitatively determined by the quantity of serum instilled and by the extent of mucous membrane with which it comes in contact.

The experiments seem to show that a few nasal instillations of serum quantitatively below a fairly definite minimum lead to a general elevation of the threshold of cellular irritability towards the antigen. Instillations of serum quantitatively in excess of a certain minimum induce, on the other hand, the opposite effect and lower the threshold of cellular irritability. In the first case, the advent into the body of an enormous increase in the amount of serum is tolerated with indifference; in the second case, a comparatively small toxic injection sets up vital reactions with a fatal outcome. Furthermore, it has been demonstrated that the direction in which the plane of metabolic irritability is shifted, and according to which the animal's sensitiveness to the antigen is decreased or increased, is determined by the first two to four of a series of separate instilla-

tions. Transferring these conceptions to the field of clinical experience, we find an explanation of many empirically determined truths.

No one will question the necessity of imposing absolute rest upon a member which has suffered an infected wound, an insistence on which has recently been made by Heidenhain.[4] The parting of the way to recovery or death is often marked by the signs of rest or use at the moment of injury. It is obvious that toxic absorption from the site of trauma must be quantitatively in somewhat inverse proportion to the quietude of the infected part.

If we apply to clinical conditions deductions drawn from our experiments with horse serum, the danger to the human organism from local infection lies not in the absorption of a lethal dosage of poison but in the fact that quantities of toxin in the circulation in excess of a certain minimum render the living cells hypersusceptible to the toxin and transform them to a state of disastrous reactivity (allergy). On the other hand, absorption of toxins in amounts below the critical minimum is not biologically indifferent, but progressively strengthens the resistance of the cellular protoplasm against the later onslaught of enormous doses of toxin. In short, we wish to indicate the necessity for rest in therapeusis.

Our experiments indicate that the elevation or depression of the plane of cellular irritability has been determined at the end of a day or two after inoculation with poisonous material; hence the peculiar value of the early application of the rest treatment. Heidenhain and others have pointed out the surpassing importance of rest at the beginning of a course of surgical infection. Abundant as is the clinical endorsement of similar treatment during the prodromal stages of all medical infections, we must express our doubt whether medical practitioners generally apprehend the value of that early quietude on the part of the patient, the therapeutic importance of which we have sought to establish on a rational basis. Medical infections, whether acute or chronic, undoubtedly involve the same principles as those set up by accident or intention. The necessity for rest and exercise as here set forth will find its effective censor in the practical clinician.

[4] Heidenhain, L., *Münch. med. Woch.*, 1915, lxii, 1482.

It may be suggested that in prophylactic vaccination, as against typhoid fever, a new importance is given to the choice of quantity of material employed in the initial dose. Finally, it must be clear that underlying these investigations is the desire to define more accurately the general principles of prophylaxis against infectious disease.

It is not improbable that choice of the nasal route as a channel of protective inoculation would be simply a return to ancient practice; it is said that the Chinese and Hindus long ago vaccinated against smallpox by blowing the powdered virus into the nose.[5] Today literature is beginning to show evidence of desultory use of the nasal mucous membrane as an avenue of inoculation against infection.[6]

We have been strengthened in the notion, suggested in our former paper, that the introduction of an antigen into the organism leads to the development of two antagonistic antibodies, one of which makes for anaphylaxis and the other against it. If this is true, it is evidently the characteristic property of nasal absorption to allow the easy propagation of one antibody in preference to the other. We think we have demonstrated the experimental conditions under which this can be done. It is a familiar fact that normal guinea pigs inoculated with the serum of hypersensitive animals become themselves passively anaphylactic. It may be proper to record here that, in a course of experiments still under way we have found, in cooperation with Mitchell, that the serum of our protected guinea pigs when inoculated into normal animals has, under certain conditions, been able to protect them from the effects of several intravenous injections of the antigen given at 14 day intervals.

SUMMARY.

1. Normal guinea pigs treated by four to six instillations of horse serum into the nose on alternate days become either hypersensitive or refractory to an intravenous injection of 0.38 cc. of serum given 16 days after the last instillation. If the amount of serum in each

[5] Klebs, A. C., *Bull. Johns Hopkins Hosp.*, 1913, xxiv, 69.

[6] See Paget, O., *Med. Rec.*, 1915, lxxxviii, 470. Herrman, C., *N. Y. State J. Med.*, 1915, xv, 233.

instillation is as much as 0.2 cc., anaphylactic death is caused by the toxic injection. If the amount of serum in each instillation is reduced to 0.04 cc. the first intravenous injection is without marked effect, and a second injection and subsequent injections of the same amount of antigen are well tolerated in about half the cases.

2. The effect produced by a given dose of serum, whether protective or anaphylactic, depends probably upon the extent of contact with the mucous membrane of the nose.

3. Guinea pigs which, after nasal treatment, have become tolerant to a definite maximum intravenous injection of the antigen appear to increase the degree of their tolerance, at least up to a resting period of more than 4 months. The same does not hold in animals immunized by the peritoneal route.

4. The first two or three instillations of a series probably determine the biologic character, whether of hypersensitiveness or hyposensitiveness, of reaction towards the serum.

5. It is probable that, contrary to the case in parenteral sensitization, hypersensitiveness and protection, respectively, set up by nasal instillations and not followed by parenteral injections, gradually disappear in about 50 to 100 days.

6. We have failed in attempts to eliminate hypersensitiveness, due to subcutaneous injection of serum, by nasal instillations which would protect the normal animal from the development of anaphylaxis.

7. It is suggested that the principles of prophylaxis evolved under these relatively simple conditions should be applied in the study of infectious disease.

CONCLUSION.

We deduce from our observations that the peculiar value of rest in the treatment of infection depends upon the fact that absorption of minimal amounts of toxic matter produces a positive protective reaction in the organism, while the absorption of larger amounts renders the cells hypersensitive. The biologic response to the intoxication is probably chiefly determined within the first 48 hours of absorption, and, therefore, rest at the beginning of an infective process has preponderant prophylactic value.