IMMUNITY IN MUMPS

V. The Correlation of the Presence of Dermal Hypersensitivity and Resistance to Mumps*

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(Received for publication, June 11, 1946)

It has been demonstrated previously that following attacks of mumps a state of specific hypersensitivity to material containing the virus may become established after varying periods of time (1). Such hypersensitivity is revealed by the mild, local inflammatory response which follows within 24 to 48 hours the intradermal inoculation of the antigen. In preliminary reports (2, 3) it has also been pointed out that most persons who were able to give a reliable history of an attack of mumps responded by skin reactions of this delayed type. On the other hand, only about one-half of those who denied previous attacks failed to react. Taken together these observations suggested that inapparent infections might frequently occur and that a positive skin reaction might be interpreted as indicative of immunity when considered in relation to the fact that known infection with the virus usually confers resistance which is enduring and effective.

Since the publication of these incomplete accounts, additional data have been obtained which, with the older observations, will be critically analyzed here and which serve in general to corroborate the earlier findings as well as to define more precisely the significance of the reaction and its limitations as an index of immunity.

Procedures

Most of the skin tests were carried out with a saline suspension of parotid gland obtained from monkeys infected *via* Stensen's duct with monkey-adapted virus. The suspension was heated at 65°C.[1] for 20 minutes and preserved with 0.5 per cent phenol. The details of the

* These investigations have been carried out as a project of the Commission on Measles and Mumps, Board for the Investigation and Control of Influenza and other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, United States Army.

We wish to express our great appreciation for the assistance of Mrs. Jeanette H. Levens and Miss Beatrice Payson without whose indispensable contributions in carrying out the technical procedures these investigations could not have been completed.

[1] Recent experiments with egg-adapted virus have shown that its infectivity for the embryonated egg is destroyed by heating for 20 minutes at 55°C. in isotonic phosphate buffer at pH 7.2.

preparation and standardization of this material as well as those concerning the preparation of the normal monkey parotid tissue employed as control have already been presented (1). Certain of the tests summarized in this paper were done with preparations which did not contain phenol as a preservative. Comparative experiments, however, have shown that the addition of phenol does not affect the results of the test, at least when the material has not been stored for prolonged periods of time. When it was found that the virus of mumps could be cultivated in the embryonated egg (4, 5), suspensions of amniotic sacs of infected chick embryos were prepared in the same manner and their activity as skin test agents compared with that of monkey gland suspensions of the same titer in respect to complement-fixing antigen. As control in these experiments a heated suspension of normal amniotic sac prepared and diluted in the same manner was employed.

In certain groups which were tested, readings were taken at 24 hours and again at 48 hours after the injection; in others the reactions were recorded only after 24 or 48 hours. Cross-diameter measurements of the area of erythema were recorded. For the sake of convenience, their mean value was recorded since in most instances the differences between the two diameters was small. Usually the redness was either moderate or marked and the margins of the involved area clearly defined; occasionally the reaction was so mild as to leave some uncertainty as to its exact extent. Careful inspection, however, nearly always enabled the observer to determine its dimensions with reasonable accuracy. Although variations in the intensity of the erythematous response were noted, they were difficult to express quantitatively and so have been disregarded here. By so doing, however, some error may have been introduced, since *a priori* it would seem probable that a very faint reaction may not have been of significance. Records were also made of the presence and degree of induration. This manifestation of inflammation was not invariably present. When it was, its intensity varied within wide limits. Nevertheless, in the large majority of clearly positive reactions some thickening of the cutis was perceptible upon palpation, although it may not have been discernible upon inspection. Again, because of the difficulty of accurately estimating the degree of induration, we have not attempted to correlate it with the immunologic status of the individual.

Complement fixation tests were frequently carried out on specimens of serum taken at various times. The technique employed has been described (6).

A number of groups of children and adults was studied. Many of these were the same as those described in the previous paper (7). For general information concerning the characteristics of each group, Table I in that communication should be consulted. We have arbitrarily considered as children those whose age was between 2 and 17 years; but the ages of the large majority of persons so classified were from 4 to 15 years. Statements in regard to previous attacks of mumps were believed to be unreliable in most of the groups, and so have not been taken into account. Those groups in which the histories were regarded as fairly dependable have been indicated in Table I of Paper IV (7).

Following mass skin testing, epidemics of mumps occurred in several of the groups. In others one or a few cases had appeared immediately before the testing was done. The very large majority of the patients were seen and the diagnosis verified by one or more of the authors; in a minority the identification of the disease was carried out by other physicians.

EXPERIMENTAL

Results of Skin Tests in Persons Who Subsequently Developed Mumps.— Sixty-five children and 24 adults in whom the skin test was satisfactorily performed[2] afterwards came down with mumps. The interval between the test

[2] Throughout this report, unless explicitly indicated, the records of individuals who reacted in any visible degree (reaction of 1 mm. or greater) to the control material have been disre-

and the appearance of parotitis in most instances varied from 1 day to somewhat more than 2 months. In a few cases 1 or 2 years elapsed before the attack occurred. In confirmation of earlier observations (1, 3) the results showed that the disease appeared most often in those who completely failed to respond to the inactivated virus. Occasionally, however, certain others exhibited erythema at the site of inoculation. Evidently, then, a reaction could not be accepted *per se* as evidence of immunity without qualification. It therefore became essential from the practical point of view to determine if possible a relationship between the size of the reaction and the resistance or susceptibility of the subject.

The distribution of the reaction size in these 89 persons which is presented in Table I provided a basis for estimating the limiting size of the reaction which may be usually associated with the susceptible state. Additional supportive data will be presented hereafter. Considering at first the combined results:

TABLE I

Skin Test Reaction Size at 48 Hours in Adults and Children Who Later Developed Mumps

Group	Mean diameter of erythema, *mm.*													
	0		1–5		6–10		11–15		16–20		21–25		>25	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Adults	16	67	0	0	3	12	2	8	1	4	1	4	1	4
Children	52	80	4	6	4	6	4	6	1	2	0	0	0	0
Combined	68	76	4	5	7	8	6	7	2	2	1	1	1	1

Of these 89 cases, about 75 per cent occurred among those who, after 48 hours, exhibited no reaction to the test material. Of the remainder who reacted, about one-half exhibited a small zone of erythema of average diameter not exceeding 10 mm. Thus approximately 90 per cent of those who later developed mumps either gave reactions of 10 × 10 mm. or less or else no response at all. In 7 per cent the reactions were between 11 and 15 mm. in diameter and in only 4 per cent were responses of greater magnitude noted. A reaction larger than 25 mm. was only once recorded.[3]

garded. This procedure has been adopted to render as unequivocal as possible the data from which conclusions have been drawn in respect to the significance of the reaction to the virus. It should be emphasized, however, that when it did occur the reaction at the control site was usually so slight that it did not interfere with the reading of the test.

[3] This occurred in a young woman who, with 11 others of similar age, responded by abnormally extensive and severe reactions accompanied by considerable pain and swelling—symptoms which have not again been encountered in many hundreds of tests. It is possible, therefore, that in this exceptional case the reaction may have been non-specific occasioned by the use of unsuitable material.

Possibly these figures, derived from the combined results obtained in adults and in children may give, in respect to the older age group, a somewhat inaccurate conception of the relation between reaction size and susceptibility. Thus only about 80 per cent of the adults as contrasted with over 90 per cent of the children exhibited either no response or reactions less than 11 mm. in diameter. The comparatively small number of adults, however, renders it impossible at present to conclude that, when susceptible, they develop more frequently larger reactions than children. This possibility should be kept in mind, however, since, as will be shown below, the average size of positive skin reactions for adults has been found to be greater than that for children.

Results of Skin Tests in the Acute Stage of Mumps —Confirmatory of these findings are the results of tests carried out during the first 5 days following the onset of mumps in 13 adults and 27 children, which are summarized in Table II. In both age groups over 90 per cent were negative at this time. Indeed, only one adult and one child gave any reaction. Moreover, the former was tested on the 5th day of the disease when possibly sensitivity had already become established as a result of the existing infection.

TABLE II

Skin Test Reaction Size at 48 Hours in Adults and Children during First 5 Days of Mumps

Group	Mean diameter of erythema, *mm.*											
	0		1–5		6–10		11–15		16–20		>20	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Adults	12	92	0	0	0	0	0	0	1	8	0	0
Children	26	96	0	0	0	0	1	4	0	0	0	0
Combined	38	95	0	0	0	0	1	3	1	3	0	0

The proportion of reactors in this group was less than that in the group which was tested before the onset. This finding might suggest that a temporary state of anergy in respect to a preexisting hypersensitivity to the virus is induced by an attack of mumps. If this were so, the results obtained in the acute stage would obviously be of little significance. That anergy analogous to the temporary suppression of the tuberculin reaction in certain infections probably does not occur is indicated, however, by the fact that among those tested after the onset 2 reactions were noted, 1 of which was observed as early as the first day following the appearance of symptoms.

The Inverse Relationship between Skin Reaction Size and Morbidity.—Although these results show clearly that the disease usually appeared in those who failed to respond or responded only weakly to the test material, they do not provide an entirely satisfactory answer to the practical question of how frequently one may expect to observe mumps among groups of exposed persons exhibiting various degrees of reaction. To obtain such information, it is necessary to compare the attack rates among groups of exposed persons whose reactions were approximately of the same magnitude. In Table III the avail-

able data have been correlated in this manner.[4] It will be seen from an inspection of the figures for each outbreak as well as from the calculations based on the combined results that the attack rate per cent decreases rapidly as the skin response becomes larger. Thus it would seem that after adequate exposure the chances, on the average, range from about 1 in 3 to 1 in 6 that a person with a negative or weak skin reaction (10 × 10 mm. or less) at 48 hours will develop signs of mumps. When the reaction is moderate (11 to 15 mm.) the chances

TABLE III

Incidence of Mumps According to Skin Test Reaction Size in Adults and Children

Group	Mean diameter of erythema, *mm.*, at 24 hrs.								Mean diameter of erythema, *mm.*, at 48 hrs.							
	0		1–10		11–15		>15		0		1–10		11–15		>15	
	T*	C‡	T	C	T	C	T	C	T	C	T	C	T	C	T	C
I	17	5	10	1	12	1	8	0	15	4	7	1	1	0	13	0
II	35	20	10	5	3	0	10	2	42	26	9	4	6	0	6	0
III§	33	12	18	6	2	0	9	0	—	—	—	—	—	—	—	—
IV	70	8	11	1	26	0	14	0	62	7	5	1	17	0	37	1
V	—	—	—	—	—	—	—	—	21	4	7	0	13	0	10	0
VI	—	—	—	—	—	—	—	—	43	25	1	0	12	4	14	1
VII	—	—	—	—	—	—	—	—	26	8	3	0	3	0	2	0
VIII	—	—	—	—	—	—	—	—	11	2	8	0	8	0	17	0
IX	—	—	—	—	—	—	—	—	24	1	9	0	9	0	27	0
X	—	—	—	—	—	—	—	—	19	3	2	2	6	1	15	1
XI	—	—	—	—	—	—	—	—	22	6	4	1	4	1	20	0
Combined	155	45	49	13	43	1	41	2	285	86	55	9	79	6	161	3
Case rate, *per cent*	30		27		2		5		30		16		8		2	
Per cent of cases in each skin test group	75		20		2		3		83		9		6		3	

* T = number tested.

‡ C = number of cases of mumps occurring in group showing skin reaction size as indicated.

§ This group consisted of boys at a preparatory school who gave negative histories of mumps.

are about 1 in 13; and when the reaction size lies between 16 and 25 mm. the chances are about 1 in 50. With a single doubtful exception, which has already been mentioned, no individual so far observed who has presented a reaction larger than 25 mm. has come down with the disease.

The figures in the last row of Table III which show the percentages which occurred in each skin test group require comment. Although they indicate that about 90 per cent appeared among those showing negative or weak reactions, this difference might not have been so great

[4] See Table I (reference 7) for composition of most of these groups.

had the numbers in each skin test group been equal. From the case rates (second row from the bottom of Table III) may be calculated the numbers of cases which might have been expected in each skin test group (48 hour readings) if the numbers of persons tested in each group had been equivalent to that of the negative group (285). The proportion of cases which might have occurred in each group would then be:

Negative	*1–10 mm.*	*11–15 mm.*	*16–25 mm.*
54	29	14	3

A comparison of these figures with those in the last row of Table III shows that the proportions of those exhibiting weak and moderate reactions who might become ill might well have been larger than those observed. Even so, over 80 per cent of the cases would still be expected to occur among individuals with negative or weak reactions and very few among those showing reactions greater than 15 mm.

One may also question the foregoing statistical conclusions based on the combined data because of certain other considerations, some of which already have been mentioned. The figures include results of tests which were done within 5 days after signs of mumps had appeared. Possibly these persons may not have been in the same immunologic status as those tested before the onset. Separate analyses of the data for each class, however, have shown that essentially the same attack rates obtain irrespective of whether the test was done before or just after symptoms appeared. The only significant difference revealed by this procedure was a decrease in the proportion of those giving weak reactions in the group with acute mumps.

The attack rates are derived from observations made in groups composed of both children and adults; but the numbers of the latter are too few to conclude that they are more likely to present what may be termed "false positive reactions," although further trials may show that a somewhat larger proportion of susceptible adults may give reactions exceeding 11 mm. in diameter (*cf.* groups X and XI which were in large part made up of adults). Since the number of children does, however, greatly exceed that of adults, one can be assured that the interpretation of the reactions as presented above would be valid in tests done on persons in the younger age group.

Finally it may be urged because of the wide divergency in the morbidity of the different groups, the intensity of exposure or some other unknown factor must have greatly varied. If so, the procedure of combining the results could be regarded as incorrect. Although it cannot be asserted that the exposure was of uniform intensity in the various outbreaks, nevertheless in each there were opportunities for frequent and intimate contact with cases in the prodromal and acute stages within the family or in dormitories of crowded institutions.

In the light, then, of the experimental data and the analysis of them which has been presented, we may assume as practical criteria for interpreting the results of the skin tests (*a*) that, in general, persons exhibiting after 48 hours reactions of less than 11 mm. may be regarded as presumptively susceptible and their skin reactions designated as negative, and (*b*) that, in general, persons exhibiting reactions 11 mm. or greater may be regarded as presumptively immune and their skin reactions designated as positive. These criteria will be employed in the evaluation of the material which will be presented hereafter. But it should be clearly understood that these statements possess only a statistical and not an absolute validity; for it is evident that after exposure a small number of individuals with reactions exceeding 10 mm. will develop mumps and conversely many of those with negative or slight reactions will apparently fail

to come down with the disease. In regard to the latter class, however, it should be borne in mind that inapparent infection may be frequent (7).

The Inverse Relation between the Attack Rate and the Incidence of Positive Skin Tests.—In Table IV certain of the results already mentioned with others obtained in groups which were exposed to mumps but in which no secondary cases occurred are presented in a manner to reveal any relationship which may have existed between the incidence of positive reactors and the attack rate.

It will be seen that when the incidence of positive reactors was small, the morbidity tended to be high. Although there is no exact quantitative relationship between the attack rate and the incidence of positive reactors, in general

TABLE IV

Mumps Attack Rates and the Incidence of Positive Skin Tests at 48 Hours

Group	No. tested	Composition		No. attacked*		Positive tests	Attack rate
		Adults	Children	Adults	Children		
						per cent	*per cent*
VII	34	8	26	1	7	15	24
II	63	3	60	2	28	19	48
VI	70	15	55	4	26	37	43
I	36	2	34	0	5	39	14
V	51	36	15	1	3	39	8
IV	121	24	97	1	8	45	7
XI	50	38	12	7	1	48	16
X	42	36	6	6	1	50	17
IX	69	68	1	1	0	52	2
VIII	44	43	1	2	0	57	5
XII	63	57	6	1	0	60	2
XIII	49	49	0	0	0	67	0
XIV	85	55	30	0	1	78	1

* Primary cases not included.

when the latter was less than 50 per cent, secondary cases were fairly numerous; when it was greater than 50 per cent, secondary cases were relatively rare events.

Although differences in the intensity of exposure, as before, may have accounted in part for the observed differences, it would seem unlikely that this factor alone was concerned. Thus there can be little doubt that the exposure in the two family groups X and XI was as intimate as that in the institutional groups II and VI and yet the attack rates of the latter were about 3 times as great. It is also possible that age *per se* may have also been involved since the number of cases which developed among the groups containing a large proportion of adults was in most instances small in contrast to the moribdity of groups in which children predominated. A comparison, however, of the number of cases in each group with the number in each age class within the group reveals no definite indication that children are inherently more susceptible to mumps than adults who have not been infected previously with the virus.

It would seem, therefore, that neither variation in exposure nor age was the principal cause of the observed differences in the attack rates. Accordingly, since epidemiologists in general recognize that an important factor in setting the case rate is the proportion of susceptible individuals in the group, this apparent inverse correlation between a high incidence of positive skin tests and low morbidity affords certain additional evidence for regarding the hypersensitive response as an index of resistance.

If these considerations be accepted, one might by means of the skin test obtain in advance a rough estimate of the number of cases likely to appear in a group which had been exposed to the disease. Such information could be of value in the management of outbreaks in institutions for children or among units of military personnel.

TABLE V

Distribution of Skin Test Reaction Size after 24 and 48 Hours in Adults and Children

Group	Readings taken	No. tested	Mean diameter of erythema, *mm.*																			
			0		1–5		6–10		11–15		16–20		21–30		31–40		41–50		51–60		61–70	
			No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
	hrs.																					
Adults.....	24	299	75	25	15	5	45	15	70	23	52	17	30	10	10	3	2	1	0	0	0	0
"	48	529	117	22	14	3	44	8	80	15	84	16	105	20	63	12	17	3	3	1	2	<1
Children....	24	349	206	59	16	5	31	10	38	11	37	11	16	5	4	1	1	<1	0	0	0	0
"	48	306	162	53	9	3	24	8	43	14	31	10	24	8	10	3	2	1	1	<1	0	0

The Range of Skin Reaction Size in Normal Adults and Children.—To procure further information which might be of assistance in the interpretation of skin reactions, the distribution of the various degrees of response after 24 and 48 hours which were observed in fairly large groups of adults and children not known to have recently had mumps was determined. The data arranged according to the mean diameter of erythema are presented in Tables V and VI.[5] The most striking feature of these tables is the higher incidence of nonreactors among the children as contrasted with the adults. It will be seen that whether the readings were taken at 24 or at 48 hours, their proportion among

[5] It should be pointed out that the results include those obtained in some of the groups previously mentioned in which mumps had broken out. The testing, however, in such cases was done within a few days after the primary case or cases had been recognized. It is extremely unlikely, therefore, that exposure under these circumstances could have in any significant manner changed the immunologic status of the individual or the group, and so we believe that these persons can, insofar as recent exposure to mumps is concerned, be regarded as normal.

the children was approximately twice as large as in the older age group. This finding, of course, might have been anticipated from the known facts concerning the age distribution of the disease, provided failure to react is indeed an index of susceptibility.[6] The proportions of weak reactors (1 to 5, 6 to 10 mm.) which, as we have shown, must also be regarded as presumptively susceptible were approximately the same in both age groups, although they might also have been expected to diverge to the same extent as those for the negative reactors. No entirely satisfactory explanation for this apparent discrepancy has occurred to us.

Although positive reactors (>10 mm.) were more frequent among adults, their mean incidence among the 529 persons mentioned in Table VI (48 hour

TABLE VI

Incidence of Negative, Weak, and Positive Skin Reactions after 24 and 48 Hours in Adults and Children

Group	Readings taken	No. tested	Mean diameter all reactions	Mean diameter reactions >10 mm.	Erythema					
					None		10 mm. or less		>10 mm.	
					No.	Per cent	No.	Per cent	No.	Per cent
	hrs.		*mm.*							
Adults	24	299	15.8	18.8	75	25	60	20	164	55
"	48	529	22.2	24.2	117	23	58	11	354	67
Children	24	349	14.2	18.3	206	59	47	14	96	28
"	48	306	16.7	20.0	162	53	33	11	111	36

readings) was only 67 per cent. This figure closely approaches the previous attack rate (64 per cent) of mumps in adults in the United States (8). But since, by means of the complement fixation test, evidence has already been adduced to show that inapparent infections are not uncommon (7), one would expect in this age group that the mean incidence of positive reactors would have been greater. Without much doubt the fact that about two-fifths of the adults tested and read at 48 hours were inmates of institutions where many of them had lived since childhood accounts in part for the low proportion of reactors. So segregated, the chances of exposure were probably less than under circumstances of normal life. Support for this hypothesis is given by the definitely lower incidence of 63 per cent positive tests found among such persons

[6] A lower incidence of positive complement fixation tests was also found among children as contrasted with adults. The mean difference, however, between these classes was not so great as that determined by skin testing. This lack of complete agreement in the results obtained by the two methods may be in part at least attributed to certain differences in the groups included in each case.

as compared with an incidence of 72 per cent recorded for the groups of medical and dental students and student nurses. Furthermore, although the mean incidence among the student groups was 72 per cent, it was found to be as high as 74 and 75 per cent respectively in one class of dental students and one class of medical students. Had the survey included a greater number of older individuals who had led essentially normal lives in urban districts, it is possible that the incidence of positive reactors would have been found to be even higher.

The second point of significance in respect to the material presented in Tables V and VI is the revelation of the fact that adults as compared with children tended to present larger reactions. This difference is evident, however, only in the 48 hour readings. At that time the incidence of reactions ranging from 20 to 40 mm. was about three times as high in the older groups. Although reactions exceeding 40 mm. were uncommon in both classes, they were nevertheless encountered about 5 times more often among the adults.

From the practical standpoint, it is of importance to note that among the adultswho gave positive tests, over 77 per cent exhibited reactions exceeding 15 mm. In routine testing, then, only about one-quarter of all positive reactions would be expected to fall within the range (11–15 mm.) where the greatest uncertainty as to the correct interpretation has been shown to exist. Even in children, on the basis of these findings, about 65 per cent of the positive reactions should be found to exceed 15 mm. in diameter.

The Greater Reliability of Readings Taken at 48 Hours.—Reference to Tables V and VI will show that in both children and adults the average diameter of reactions was smaller at 24 hours than at 48 hours. As a result of the tendency of the response to become more marked during this interval, the proportions of negative and weak reactions are diminished at 48 hours. In the adult groups, negative reactions are reduced by 3 per cent and weak reactions by about 10 per cent. These reductions are reflected in a correspondingly larger proportion of reactions exceeding 10 mm. at 48 hours. Clearly these comparisons reveal the greater accuracy of the 48 hour reading.[7]

Reference to Table III will further support the selection of the 48 hour reading as the most reliable. There it will be seen that the attack rate was significantly higher among those exhibiting weak reactions at 24 hours, whereas among the negative reactors it was the same irrespective of the time at which the readings were recorded. Furthermore when the readings were made at 24 hours the incidence of mumps was higher in the group showing reactions exceeding 15 mm. The only group in which the 24 hour interval seemed to afford a more accurate index of resistance was that in which the reactions were 11 to 15 mm. Since the numbers tested were small compared with those of the corresponding 48 hour group, it would be unwise in this single instance to stress the apparent advantage of the 24 hour reading.

[7] The mean reaction sizes are not derived, as ideally they should be, from measurements made after both intervals in the same individuals. This departure from the ideal procedure was occasioned by our uncertainty during the earlier part of the study, before many tests had been performed, as to the optimal period which should elapse before the reading was taken. But it would seem that little error has been introduced by treating the data in this manner since a comparison of the results of tests carried out in over 200 adults in whom readings were taken both after 24 and 48 hours has yielded differences of approximately the same magnitude.

For these reasons, then, we consider the character of the reaction after 48 hours to reflect most accurately the hypersensitive state of the individual and accordingly have employed it wherever possible in correlating the results of skin testing with those of the complement fixation test and histories of the disease which will be subsequently presented.

Reaction to Control Material (Normal Monkey Parotid Gland).—From still another point of view the 48 hour reading offers a definite advantage. Skin tests on many hundreds of individuals have shown that a small but varying proportion develop erythema at the site of the inoculation of the control material. As examples the percentages of individuals showing control reactions at 24 and 48 hours among 288 adults and 186 children are presented in Table VII. In the adults, it is clear that reactions occurred much more frequently at the control site at 24 hours. Thus 37 persons who reacted at 24 hours showed no reaction to the control material at 48 hours. In contrast, 5 only proved negative at 24 hours and subsequently developed a reaction at 48 hours. As a result of this tendency of most control reactions to decline in intensity or become negative after the longer interval, only about 4 per cent of all the adults tested pre

TABLE VII

Reactions to Suspensions of Normal Monkey Parotid Gland (Control Material) in 474 Adults and Children

Group	No. tested	Reacted to control		Mean diameter of reactions		No. positive at 24 hrs., negative at 48 hrs.	No. negative at 24 hrs., positive at 48 hrs.
		24 hrs.	48 hrs.	24 hrs.	48 hrs.		
		per cent	*per cent*	*mm.*	*mm.*		
Adults	288	15	4	11	10	37	5
Children	186	3	<1	8	7	4	0

sented any control reaction at 48 hours. Furthermore, because certain of the control reactions at 48 hours were much smaller than the reaction to the heated virus, from the practical standpoint, it was not difficult to decide whether or not the reaction was to be designated as positive or negative. Indeed among these adults there were only 2 instances in which the reaction at the site of the control interfered with the interpretation of the test.

Turning now to the incidence of control reactions among the groups of children, one would be inclined to say that their tendency to react non-specifically is clearly less than that of adults, but a comparison of 2 of the individual groups of adults included among the 288 mentioned in Table VII with the children's group as a whole has not revealed a significant difference in the incidence of control reactions. In certain groups the apparent difference in response of adults as compared with children may have depended rather upon differences in the properties of the various preparations of normal monkey parotid which were employed than upon physiologic factors related to age. An analysis of our results shows that occasionally this material may develop irritative properties after standing in the ice box. The nature of the substances which may cause irritation is entirely unknown. But these observations clearly point to the necessity for care in the preparation, testing, and preservation of the reagents used in this test.

The Effect of Dilution of the Skin Test Material on the Size of the Reaction.—In an experiment carried out in a group of medical students in which two dilutions of the inactivated virus were employed in order to determine the effect of decreased concentration of antigen on the skin response, it was again clearly demonstrated that the maximum reaction occurred at about 48

hours. The results also are of importance in respect to the technique which has been described for the standardization of the material employed in the test (1). The complement-fixing titer of the monkey gland suspension used was 1–450. Dilutions of 1–30 and 1–240 of this preparation were inoculated into 110 individuals. Seventy-four responded with some reaction at the site of inoculation of both dilutions at 24 hours and 72 still showed reactions at the same sites after 48 hours. In none of these were any reactions observed at the site of the inoculation of the control material (1–30 normal parotid gland suspension). The mean diameters at 24 hours for the stronger and weaker suspensions of inactivated virus were 14 and 10.4 mm. respectively whereas after 48 hours the mean diameters were 23 and 15.3 mm. respectively. The lower concentration of antigen, then, gave at 24 hours a mean diameter which was less than that regarded as indicative of a positive test, but after the longer interval the mean diameter was definitely within the range of reactions which we have regarded as indicative of a positive test with the standard antigen.

Although the concentration of antigen in the 1–240 dilution was only 1/8 that of the 1–30 dilution, the mean diameter of reaction it induced was about 2/3 as large as that produced by the standard dose. Moreover, there were only 16 individuals out of 74 whose reactions to the diluted material would not have been interpreted in the same sense as those induced by the standard. These results, therefore, afford some support for the procedure which has been followed in the standardization of the skin test material.

Comparison of Infected Monkey Parotid Gland and Infected Amniotic Membrane as Antigens for Testing Dermal Hypersensitivity.—At this point it is convenient to consider the data derived from experiments in which the reactions following the injection of the standard gland suspension were compared with those occurring in the same individuals after inoculation of suspensions of infected amniotic membrances. It has recently been shown that from the amniotic membrane good yields of complement-fixing antigen are regularly secured following the inoculation of the virus of mumps into the amniotic cavity of embryonated hen's eggs (5). Because of the high concentration of antigen in this tissue, and because of its apparent freedom from yolk material and egg albumin, it appeared to us to present the most favorable attributes of all the embryonic components for preliminary trial as a skin test antigen. In these experiments the concentrations of complement-fixing antigen in egg and monkey materials were rendered equivalent.

The results of skin tests carried out in 59 "normal" adults and 23 children in which these antigens were employed are summarized in Table VIII. It is evident that on the average the monkey antigen gave larger reactions at both 24 and 48 hours. Indeed, in the adults most of the reactions to the amniotic membrane measured less than 11 × 11 mm. at 48 hours. Moreover, among this group at least, the monkey material much more frequently than the membrane produced positive reactions. But in 4 children the membrane gave at 48 hours a positive test when the monkey antigen either induced no reaction or one less than 10 mm. in diameter. Nevertheless when taken together these results would seem to indicate that on the whole the monkey material is a more satisfactory testing agent. The use of monkey material appears also to be of

advantage because of the fact that it has given rise to fewer non-specific responses as compared with the amniotic membrane. Thus among 49 individuals in whom simultaneous tests were carried out with both kinds of antigen, 4 reacted to the monkey control at 24 hours and 2 at 48 hours. All these reactions were less than 10 mm. In contrast, 28 reactions to the membrane control were noted at 24 hours and 27 at 48 hours. At the latter time, however, all but one of the reactions were less than 10 mm.

Habel (4) has previously reported the results of comparative experiments in which yolk sac and presumably amniotic sac and allantoic sac materials[8] were employed together with monkey

TABLE VIII

Comparison of the Activity of Infected Monkey Parotid Gland and Amniotic Membrane of the Chick Embryo as Skin Test Antigens

Group	No. tested*	Reactions to gland				Reactions to membrane				No. positive to gland, negative to membrane§		No. positive to membrane, negative to gland§	
		24 hrs.		48 hrs.		24 hrs.		48 hrs.					
		No.	Diameter‡	No.	Diameter	No.	Diameter	No.	Diameter	24 hrs.	48 hrs.	24 hrs.	48 hrs.
Adults group XV.....	59	44	12.5	49	16.	47	10.	55	10.9	11	20	3	1
Children from group I..	11	2	14.	3	17.	3	14.3	3	9.3	1	2	1	1
Children from groups VI and VII.....	12	nr‖	nr	9	16.5	nr	nr	11	14.8	nr	0	nr	3
Combined.....	82	46	12.6	61	16.1	50	10.3	69	11.4	12	22	4	5

* Includes in the various groups tested only those who gave no control reactions to either normal gland or membrane suspensions.

‡ Mean diameter (mm.).

§ Reactions >10 mm. taken as positive; those <10 mm. as negative.

‖ nr = not read.

gland suspension in 58 individuals, 14 of whom were children. The egg materials gave specific reactions in every instance in which the monkey suspension yielded a positive test. In a rather large number of cases, however, reactions were obtained with the egg and not with the monkey antigen. Detailed information in respect to the size of the reactions was not presented. Because of these discrepancies between Habel's results and ours and because the total number of individuals studied is small, further investigation in which the amniotic membrane as well as other constituents of the egg is tested will be necessary before any final conclusions can be drawn in respect to the comparative merits of egg and monkey antigens.

Correlation of the Results of Skin Testing with History of Mumps.—

(*a*) *Adults.*—From the facts so far established in this and earlier communica-

[8] It is impossible from his data to be certain whether these tissues or the corresponding fluids were used.

tions (1) concerning the development of hypersensitivity following observed attacks of the disease and the correlation of positive reactions with resistance to subsequent exposure, it might be anticipated that persons who at some time in the past have suffered an attack should in the very large majority of cases give positive skin tests. Furthermore a significant proportion of those in whom the disease was not clinically recognized should also react positively, since it has been demonstrated by the serologic method that inapparent infections are not uncommon under epidemic conditions (7).

TABLE IX

Correlation of Previous History of Mumps and Skin Test Reaction Size at 48 Hours in 311 Adults

Group	No. tested	History	Mean diameter all reactions	Mean diameter reactions >10 mm.	Erythema					
					None		10 mm. or less		>10 mm.	
					No.	Per cent	No.	Per cent	No.	Per cent
			mm.							
XIII	25	Negative	23.5	25.8	11	44	2	8	12	48
XV	27	"	14.7	18.6	5	19	7	26	15	56
XVI	17	"	16.0	17.4	3	18	2	12	12	71
XVII	40	"	15.9	18.9	11	28	8	20	21	52
XVIII	3	"	21.6	21.6	0	0	0	0	3	100
XIX	4	"	25.1	25.0	3	75	0	0	1	25
XX	16	"	21.2	21.2	2	13	0	0	14	88
Combined...	132	Negative	18.6	20.3	35	27	19	14	78	58
XIII	24	Positive	27.0	27.0	3	13	0	0	21	88
XV	50	"	16.7	19.2	5	10	9	18	36	72
XVI	30	"	18.6	20.8	3	10	5	17	22	73
XVII	64	"	27.0	27.7	5	8	2	3	57	89
XVIII	9	"	29.2	29.2	0	0	0	0	9	100
XIX	2	"	26.0	26.0	0	0	0	0	2	100
XX	0	"	0	0	0	0	0	0	0	0
Combined...	179	Positive	22.9	24.6	16	9	16	9	147	82

The data presented in Table IX support these deductions. The various groups of adults selected for presentation, because of the relatively great confidence one may place in their histories, consisted almost entirely of medical and dental students and student nurses. From the table it is evident that in 147 or 82 per cent of the 179 individuals whose histories were positive the skin reaction at 48 hours exceeded 10 mm. Most of those who so reacted exhibited zones of erythema greater than 15 mm.—a fact which is reflected in their mean reaction diameter of 24.6 mm. About 9 per cent failed entirely to respond and in an equal proportion reactions less than 10 mm. were observed.

Although these results taken as a whole reveal a high correlation between positive history and hypersensitivity to the virus, it is perhaps not quite as complete as might be anticipated. But examination of the data for each group will show that the average diameter of the positive reactions in groups XV and XVI was less than that recorded in the others. This strongly suggests that for some reason which is not understood the material employed in these two groups was not as potent as that used in the others. If this were so, a larger proportion of subliminal reactions should also have been encountered, and indeed it will be seen from the table that in groups XV and XVI the numbers of reactions of this sort were relatively high. If, then, one omits these two student groups, it is found that 90 per cent of the 99 remaining individuals who gave positive histories responded with reactions exceeding 10 mm. It is probable that this degree of correlation approaches the maximum which can be obtained since the tests were performed with material of apparently high potency in those whose histories were possibly as reliable as can ordinarily be secured in a group of a size sufficient for adequate statistical treatment.

From Table IX it can be seen that the mean incidence of positive skin tests was substantially lower among those who denied a previous attack, although in two of the individual groups the differences are scarcely significant. Thus on the average only a little more than one-half of those giving negative histories were found to be skin test-positive. This significantly lower rate of reactors among those giving negative histories can be regarded as further evidence in respect to the specificity of the reaction and so in turn it may be considered as additional evidence for the occurrence of frequent subclinical infections.

If we again omit the same groups concerning which there may be doubt as to the maximal potency of the skin test material and recalculate the means for those with negative histories which remain, the proportions of negative and positive reactors are found to be as follows: 58 per cent positive; 31 per cent no reaction; 11 per cent reactions <11mm. In the case, then, of these selected groups in which we believe optimal experimental conditions prevailed, the difference in the incidence of positive skin tests between those giving positive and those giving negative histories was 32 per cent.

It is also of interest to note in connection with the data assembled in Table IX that the mean diameter of the positive reactions for the groups with positive histories (24.6 mm.) is somewhat greater than that for those with negative histories (20.3 mm.) although both are within the range where it has been shown that the correlation with resistance is high. One might possibly infer from this fact that an overt attack is more apt to establish a higher level of sensitivity.

Attention should also be called to the fact that the incidence of weak reactions was usually greater among those with negative histories. The difference was particularly striking in group XVII. This finding is clearly compatible with the facts already presented concerning the frequency of mumps among exposed persons who exhibited this degree of reaction.

(*b*) *Children.*—The number of skin tests which can be correlated with histories is unsatisfactorily small among the children who have been studied. Three factors are responsible for this situation. In the first place, most of the children were inmates of institutions or day nurseries and it was felt that in many instances the histories were quite undependable. Therefore, many have been omitted, and, even in the case of the groups mentioned in Table X,

in which the histories are believed to be fairly reliable, it should be recognized that a considerable error may have been introduced through the inclusion of some with incorrect histories. Because of the low previous attack rate of mumps among children, especially of the younger individuals, very few with positive histories among those deemed suitable for inclusion on other grounds

TABLE X

Correlation of Previous History of Mumps and Skin Test Reaction Size in Children

Group	No. tested	History	Readings taken	Mean diameter all reactions	Mean diameter reactions >10 mm.	Erythema					
						None		10 mm. or less		>10 mm.	
						No.	Per cent	No.	Per cent	No.	Per cent
			hrs.	*mm.*							
XXI	11	Negative	24	12.5	15.3	7	64	1	9	3	27
XXII	15	"	"	14.0	15.7	7	47	2	13	6	40
XXIII	13	"	"	4.8	0	9	69	4	31	0	0
XXIV	15	"	"	20.4	27.7	10	67	1	7	4	27
Combined....	54	Negative	24	13.5	18.0	33	61	8	15	13	24
XXIII	11	Negative	48	7.0	0	10	92	1	9	0	0
XXIV	17	"	"	14.5	14.5	13	77	0	0	4	24
Combined....	28	Negative	48	13.0	14.5	23	82	1	3	4	15
XXI	2	Positive	24	16.0	16.0	0	0	0	0	2	100
XXII	1	"	"	18.0	18.0	0	0	0	0	1	100
XXIII	5	"	"	14.5	17.0	1	20	1	20	3	60
XXIV	3	"	"	13.0	13.0	0	0	0	0	3	100
Combined....	11	Positive	24	14.7	15.6	1	9	1	9	9	82
XXIII	5	Positive	48	22.0	26.7	1	20	1	20	3	60
XXIV	3	"	"	20.7	20.7	0	0	0	0	3	100
Combined....	8	Positive	48	21.4	23.7	1	13	1	13	6	75

were encountered. Finally, many of the reactions of the children tested were read only at 24 hours because the studies were carried out before the 48 hour reading was recognized as being more accurate. Some of these results are here recorded, however, in order to compensate in part for the small number of 48 hour readings.

As far as they go, the results in children with positive histories are roughly comparable to those in adults; *i.e.*, from 70 to 80 per cent of the reactions

exceeded 11 mm. Contrasting quite sharply with the adult groups, however, is the much closer agreement between negative test and negative history. From Table X it will be perceived that on the basis of the 24 hour readings about 75 per cent of the 54 children studied were negative. Of the 28 children tested at 48 hours about 85 per cent could be designated as negative reactors. This finding, in respect to children with negative histories, might have been predicted on epidemiologic grounds since the previous attack rate of any group would be expected to vary directly with the age of the individuals of which it was composed, and the attack rate of inapparent infection should clearly be a function of the attack rate of overt infection. It follows, then, that in children of 3 to 5 years of age who largely made up the groups mentioned in Table X the skin test should be negative in a very large proportion of those with negative histories.

Correlation of the Results of Complement Fixation Tests and Skin Tests.—

(*a*) *Adults.*—It has been shown in the previous communication (7) that on the average 77 per cent of the sera of normal adults giving positive histories contain complement-fixing antibody. On the other hand, only about 42 per cent of the sera of adults with negative histories were found to contain antibody. Because of the closer correlation of positive skin test with positive history which has been demonstrated, it becomes of interest to compare the results of complement fixation tests with those of skin tests which have been carried out in essentially the same groups of people. By so doing a quantitative estimate of the greater sensitivity of the skin test in the revelation of past infection should be obtained.

In Table XI are summarized such data derived from a study of 278 adults most of whom were included in the groups in which history and skin test were correlated. Considering first the combined results, it is evident that 87 per cent of the 167 persons whose complement fixation tests were positive gave reactions exceeding 10 mm. If, as before, the groups are omitted whose average reactions were significantly smaller than the mean size of positive reactions for all groups, it can be calculated that about 93 per cent of the remaining individuals gave positive skin tests. It may therefore be concluded that by the simpler procedure of skin testing nearly all adults can be readily discerned who would be found to have antibody in their serum by means of the more laborious technique of complement fixation.

The skin test would also appear to be more useful in the discrimination of the resistant from the susceptible because of its greater sensitivity in most instances as compared with complement fixation. This is clearly demonstrated by the results obtained in the individuals in whose sera no antibody could be demonstrated. Thus 57 of 111 such individuals exhibited positive skin reactions. This proportion of reactors, *i.e.* 50 per cent, remains the same even if the groups showing on the average weaker skin responses be omitted.

(*b*) *Children.*—In Table XII similar comparative data are given for 148 children whose skin tests were read at 48 hours. The means reveal the same general relationships but the correlation between positive complement fixation and positive skin test is lower. The factors responsible for this are not immediately obvious. The hypothesis may be invoked that children are less apt to exhibit hypersensitivity of a degree entirely comparable to that of adults. This

TABLE XI

Results of Complement Fixation Tests and Skin Tests after 48 Hours in 278 Adults

Group	No. tested	C.F.* test	Mean diameter all reactions	Mean diameter reactions >10 mm.	Erythema					
					None		10 mm. or less		>10 mm.	
					No.	Per cent	No.	Per cent	No.	Per cent
			mm.							
XIII	25	Negative	25.9	25.9	9	36	0	0	16	64
XV	36	"	15.8	20.0	10	28	8	22	18	50
XVII	33	"	19.0	24.0	13	39	6	19	14	42
XVIII	0	"	0	0	0	0	0	0	0	0
XIX	2	"	0	0	2	100	0	0	0	0
XX	6	"	20.8	20.8	2	33	0	0	4	67
XXV	9	"	20.1	23.4	3	33	1	11	5	56
Combined...	111	Negative	19.6	22.8	39	36	15	14	57	52
XIII	19	Positive	24.8	25.8	2	11	1	5	16	84
XV	54	"	16.0	18.6	2	4	11	20	41	76
XVII	72	"	24.4	25.4	2	3	3	5	67	93
XVIII	6	"	25.7	25.7	0	0	0	0	6	100
XIX	3	"	25.7	25.7	0	0	0	0	3	100
XX	5	"	18.0	18.0	0	0	0	0	5	100
XXV	8	"	28.1	28.1	0	0	0	0	8	100
Combined....	167	Positive	21.8	24.2	6	4	15	9	146	87

* C.F. = complement fixation test.

assumption would account for the larger proportion of failures to react to the skin test in the face of antibody in the serum, and it receives some support from the relatively large proportion of subliminal reactions which were recorded in two of the groups included among those with positive fixation tests. It is also possible that some of the children had suffered inapparent infections so recently that they had not become hypersensitive at the time the testing was done, although antibody had begun to appear in their sera. It is possible, therefore, that the accuracy of the skin test in revealing the presumptively immune individual is not so great in younger children as in older individuals.

But before this possibility can be finally established as a fact, further experiment will be necessary in children who are known not to have had recent opportunities of exposure.

As might have been predicted from epidemiologic considerations, the incidence of positive skin tests among the children who gave negative complement fixation tests was lower than that determined for the corresponding adult group. This is particularly striking among the youngest children (groups XXIII and XXIV). It would seem logical to account, at least in part, for the closer correlation between negative skin and fixation tests in children on the basis of the lower previous attack rates of both overt and inapparent in-

TABLE XII

Results of Complement Fixation Tests and Skin Tests after 48 Hours in 148 Children

Group	No. tested	C.F. test	Mean diameter all reactions	Mean diameter reactions >10 mm.	Erythema					
					None		10 mm. or less		>10 mm.	
					No.	Per cent	No.	Per cent	No.	Per cent
			mm.							
I	15	Negative	16.4	22.3	10	67	2	14	3	20
IV	72	"	16.5	17.8	42	58	4	6	26	36
XXIII	11	"	7.0	0	10	91	1	9	0	0
XXIV	7	"	13.0	13.0	6	86	0	0	1	14
Combined...	105	Negative	16.1	18.2	68	65	7	7	30	29
I	14	Positive	17.4	19.5	1	7	3	21	10	72
IV	13	"	20.8	21.8	1	8	1	8	11	85
XXIII	4	"	22.0	26.7	0	0	1	25	3	75
XXIV	12	"	17.8	17.8	6	50	0	0	6	50
Combined...	43	Positive	19.2	20.7	8	18.6	5	12	30	70

fection in this age group, although the possibility of diminished capacity to respond allergically must again be considered.

From the standpoint, then, of routine testing for susceptibility the skin test is the method of choice. But in younger children and, in special instances, in older individulas where the greatest possible accuracy is desired, both procedures should be invoked since at times—particularly in children—antibody may be found in the absence of dermal hypersensitivity. This situation we know can exist soon after recovery from mumps when it is usual to find antibody before the hypersensitive state has been established (1). There is no reason to doubt that following inapparent infection the same phenomena occur, so that in those who have been tested after an exposure which has taken place

within 3 weeks to 3 months, skin testing may not infrequently fail to reflect the resistant state.

The Appearance of Complement-Fixing Antibody Following Skin Testing.— The effect of the intradermal inoculation of heat-inactivated virus on the level of complement-fixing antibody has been studied in 2 groups. One of these consisted of medical students (group XV) and the other of institutionalized persons of whom about four-fifths were children (group IV). No exposures to mumps were known to have occurred in the first group shortly before or during the period of observation. A case of mumps, however, developed among the institutionalized group about 2 weeks after the sera for the second complement fixation test were drawn. Thereafter 8 secondary cases of mumps appeared.

TABLE XIII

The Capacity of Heat-Inactivated Virus (Skin Test Material) to Stimulate the Formation of Complement-Fixing Antibody

Group	Number		Number C.F. positive		Number increasing C.F. titer‡	Per cent increasing C.F. titer‡	Mean increase C.F. titer
	ST+*	ST−	1st test	2nd test			
XV	31		14	30	30	97	25 ×
"		21	4	10	10	48	15 ×
IV	41		2	39	39	96	8 ×
"		55	2	24	24	44	8 ×

* ST = skin test.

‡ Individuals showing an increase in titer by the second test as well as those in whose sera antibody was first demonstrated by the second test are included. In the case of the latter, the antibody titer of the second test was taken as the numerical expression of the increase in antibody.

These time relations indicate that there was little likelihood that the results of the second complement fixation tests were influenced by inapparent infections. But the subsequent development of this small intramural epidemic did, as we shall see, afford an opportunity for obtaining some evidence as to the possible immunizing effect of the skin test. Sera for complement fixation tests were obtained a few days before the skin test was administered and again 14 or 15 days afterwards. The endpoints of the second specimens were determined only for those obtained from group XV. In Table XIII the serologic results have been correlated with those of the skin test. It is immediately apparent that almost all the positive skin reactors either developed an appreciable quantity of antibody following the skin test or exhibited increases in the amount present before the test was performed. Because of this almost universal response, it would seem logical to regard it in such cases as a recall or "booster" effect produced by the inoculation of the inactivated virus in a person who had

previously been infected. This interpretation obviously is suggested by the conclusions already drawn in respect to the significance of the positive skin reaction. Contrasting sharply is the much lower incidence of antibody production among those with negative skin tests—less than one-half of whom responded. Again this behavior is in accord with the hypothesis that a negative reaction is obtained in persons who have not been infected with the virus inasmuch as such people in all cases would not be expected on general immunologic grounds to produce antibody following the single inoculation of what must be a very small quantity of antigen.

The fact, however, that an appreciable number of negative skin reactors did so respond indicates that virus heated at 65°C. for 20 minutes still remains antigenic and so capable of acting as a primary stimulus to antibody formation. For in the light of all the evidence so far presented, most of the negative skin reactors must be regarded as susceptible; *i.e.*, as not having had previous physiologic contact with the virus.

It should be pointed out that the quantities of antibody produced were in many cases considerable. The mean titer of 31 sera from the skin-positive medical students (group XV) was 1–108; 2 individuals whose first complement fixation tests were negative yielded titers of 1–384 on the second test. These are well within the convalescent range. Titers of 1–192 and 1–96 were recorded for 19 other students. Although among the skin test–negative students they were on the average somewhat lower, titers of 1–92 were not uncommon.

The Possible Immunizing Effect of Skin Testing.—As stated above, 9 cases of mumps appeared in the group of institutionalized persons following the second series of complement fixation tests. One of the cases was in an individual who had exhibited a moderate skin reaction, the remainder occurred among the skin test–negative group. Of these 8 cases, 7 developed among those who had failed to produce antibody as a result of the skin test. The attack rate, then, in the negative skin reactors who showed antibody responses was 4 per cent and in those who did not respond 23 per cent. These results suggest that when the skin test is followed by an antibody response, resistance may be increased. Certainly if one disregards the skin test readings of all the institutionalized group and simply determines the attack rates in those who responded by antibody formation and in those who did not, the percentage difference becomes definitely significant. Thus of 96 individuals skin tested, 63 showed an antibody response and 33 did not. The difference in the attack rates is 22.4 per cent which is over 4 times the standard error of the difference. In view of the fact, however, that most of the skin-positive reactors must be considered as immune, adoption of this method of determining significance should be viewed with skepticism. But it is, perhaps, suggestive that the only person who developed mumps among the positive skin reactors was one of two among 42 who failed to exhibit an antibody response.

We cannot conclude, therefore, on the basis of these scanty data that the

intradermal inoculation of inactivated virus induces immunity. They do, however, point to this possibility which is supported by the fact just established that the procedure is surprisingly effective in eliciting an antibody response. Additional suggestive evidence for a prophylactic effect is provided by the results obtained in certain experiments on the effects of vaccine presented in the following communication (9).

SUMMARY

The results of skin tests read at 48 hours on several hundred adults and children in which heat-inactivated mumps virus was the antigen have been presented and discussed. They can be summarized as follows:—

Of 89 persons tested before the onset of mumps, 89 per cent exhibited erythematous reactions 10 mm. or less in diameter and 95 per cent, reactions 15 mm. or less in diameter.

Of 40 persons tested during the first 5 days of mumps, 95 per cent exhibited reactions 10 mm. or less and 98 per cent reactions 15 mm. or less.

Of 480 exposed persons the attack rate of mumps was 46 per cent among 340 with reactions 10 mm. or less and 10 per cent among 240 with reactions greater than 10 mm. The attack rate was only 2 per cent among 161 with reactions exceeding 15 mm.

The attack rates in 13 skin-tested groups which were exposed to mumps tended to be inversely proportional to the incidence of reactions exceeding 10 mm.

The incidence of reactions exceeding 10 mm. was approximately twice as high among 529 adults (persons 18 years or older) as it was among 306 children (persons under 18 years).

Of 179 adults giving positive histories of mumps, 82 per cent exhibited skin reactions exceeding 10 mm. In certain groups the correlation between history and positive skin test was as high as 0.9. Of 132 adults giving negative histories, 58 per cent exhibited skin reactions of this magnitude. The proportion of reactions exceeding 10 mm. in a small number of children giving positive histories was 75 per cent. The proportion of reactions less than 10 mm. was 15 per cent.

Of 167 adults with positive complement fixation tests, 87 per cent exhibited skin reactions exceeding 10 mm. Of 111 adults with negative complement fixation tests, 52 per cent exhibited reactions exceeding 10 mm. Of 43 children with positive complement fixation tests, the skin test reactions exceeded 10 mm. in 70 per cent. The skin reactions exceeded 10 mm. in 29 per cent of 105 children with negative complement fixation tests.

In 69 of 72 individuals in whom skin reactions exceeded 10 mm., complement-fixing antibody either appeared in the blood or increased in amount within about 2 weeks after the tests were done. Such antibody responses likewise

were observed in 34 of 76 individuals in whom skin reactions were 10 mm. or less. The data summarized up to this point were obtained with virus derived from the infected parotid gland of monkeys.

The results of simultaneous tests in 82 individuals employing materials prepared from infected monkey parotid gland and amniotic membrane of chick embryos infected with mumps virus indicated in general that the same individual responded in a similar manner to both antigens. In many instances, however, the membrane material produced weaker reactions. Occasionally an individual failed to react at all to one of these materials but did respond to the other.

CONCLUSIONS

On the basis of these findings, the following conclusions have been drawn:

1. Persons exhibiting erythematous dermal reactions exceeding 10 mm. in mean diameter 48 hours after the inoculation of a suspension of heated, inactivated mumps virus obtained from the parotid gland of infected *rhesus* monkeys may be, from the practical standpoint, regarded as resistant to mumps. According to this criterion, an error in interpretation of about 10 per cent may be made. This error will be reduced to approximately 2 per cent if a reaction larger than 15 mm. be taken as the criterion for the resistant state.

2. Preliminary tests employing skin test material prepared from the amniotic membranes of infected chick embryos show that it also exhibits the capacity to induce local reactions in individuals hypersensitive to the virus or its products.

3. In general the attack rate of mumps in exposed groups will be low when the incidence of positive skin reactors exceeds 50 per cent. The skin test might, therefore, be employed to obtain an estimate of impending morbidity in family groups or units of institutionalized or military personnel.

4. By means of the skin test, additional evidence has been obtained which indicates that subclinical attacks by the virus of mumps are frequent, accounting in young adults for about 33 per cent of past infections.

5. The skin test in adults is in most instances a more sensitive indicator of past infection and hence of immunity than is the complement fixation test.

6. The skin test material is antigenic, giving rise to the formation or increase of specific complement-fixing antibody.

7. It is possible, though not demonstrated, that skin testing may lead to increased resistance to infection by the virus.

8. From a comparison of certain general epidemiologic features of mumps with those of other diseases in which the phenomenon of "population immunity" is already established, the findings reported here are in accord with epidemiologic expectancy.

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