REACTIONS OF MONKEYS TO EXPERIMENTALLY INDUCED INFLUENZA VIRUS A INFECTION*

An Analysis of the Relative Rôles of Humoral and Cellular Immunity under Conditions of Optimal or Deficient Nutrition

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Most of our current knowledge of experimental influenza has been derived from the use of small laboratory animals. Smith, Andrewes, and Laidlaw (1) first showed that the ferret, and later, simultaneously with Francis (2, 3), that the mouse is susceptible to the influenza virus. More recently Stuart-Harris (4) has described influenza infection in the hedgehog, as well as inapparent infections in the rat and guinea pig (5). The hog has been shown by Shope and Francis (6) to be sensitive to the human influenza virus.

The reaction of the monkey to experimental influenza infection was first reported by McIntosh and Selbie (7) who described a late febrile response during the 3rd and 4th week following the inoculation of a *cercopithecus* monkey. Vieuchange (8) reported certain manifestations of infection in two monkeys (*Macaca mulatta*) inoculated with an unfiltered mouse lung suspension of the "W.S." strain of virus. No appreciable temperature rise or other signs of illness were apparent in one animal, while the other showed a slight fever and some dyspnea between the 7th and 10th days. Neutralizing antibodies appeared between the 10th and 16th days. Burnet (9) has reported an induced respiratory infection in *Macaca irus* with intratracheal, but not with intranasal, inoculations of influenza virus.

A preliminary report (10) of the response of *Macaca mulatta* to intranasal inoculation with the virus of influenza was made from this laboratory in 1941. Since the reactions in the monkey can readily be followed and interpreted from laboratory data analogous to those observed in the human disease, these earlier observations have been extended, and are here reported in some detail. Simultaneous obvious, hematologic, and immunologic data were obtained and correlated.

Materials and Methods

Virus.—The PR8 strain of influenza virus A was propagated by intranasal passage in young white mice. The inoculum for the monkeys was prepared as follows: The lungs of infected mice were triturated in 9 volumes of buffered saline; the suspension was centrifugated at 1000

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R.P.M. for 10 minutes (to throw down the gross particulate matter), filtered through Berkefeld V candles, and stored at −78°C. After a period of stabilization, the virus was titrated in mice and periodically retested at intervals for virulence during the period of experimentation.

For the experiments involving the use of inactivated virus, stock suspensions as used in the basic studies were heated at 70°C. for 90 minutes. Complete inactivation of the virus was determined by means of two negative intranasal mouse passages.

Monkeys.—Healthy young monkeys (*Macaca mulatta*) were selected, isolated, and observed for a preliminary period of 2 to 3 weeks, during which time base lines for the various determinations were established. Animals meeting satisfactory base line standards were then inoculated intranasally, while under deep ether anesthesia, with 3 cc. of a saline suspension of mouse lung filtrate containing approximately 10,000 M.L.D. for mice. The animals were then observed daily for a period of 3 weeks and periodically thereafter up to 1 year.

Observation of Animals.—The monkeys were observed closely for any obvious signs of infection. Rectal temperatures were taken daily. Complete serial blood studies were made on specimens obtained from the ear veins.

Serum derived from blood drawn at 5 day intervals from the saphenous vein and stored in sterile rubber-stoppered vials, was used for the testing of antibody content. The neutralizing antibody titers against influenza virus were determined by the mouse protection test in which the serum dilution was kept constant while tenfold dilutions of virus were added in equal volume. Thus the final dilution of serum was 1 in 10, while the dilutions of virus were 1 in 10, 1 in 100, and 1 in 1000. White mice between 4 and 6 weeks of age and weighing approximately 12 to 16 gm. were used as the test animals. They were placed under ether anesthesia, and then inoculated intranasally with approximately 0.05 cc. of the serum-virus mixtures which had previously been incubated for 1 hour at 37°C. and then kept another hour at 4 to 6°C. Each serum sample was tested in nine mice for neutralizing antibodies, three mice being employed for each of the three serum-virus dilutions. Controls included mice that received the three dilutions of virus and others inoculated with a mixture of the virus and immune rabbit serum. The mice dying were necropsied and the lungs examined for signs of specific infection. Mice that survived 10 days were sacrificed and the lungs cultured, examined for lesions, and graded on the basis of percentage of consolidation.

Response of Macaca mulatta to Primary Influenza Virus Infection

Twenty-eight monkeys were inoculated with influenza virus alone, simultaneously with *Streptococcus hemolyticus*, or in varying sequences with *Streptococcus hemolyticus* and inactivated virus. Ten animals were initially inoculated with the virus alone, and were subsequently observed for manifestations of uncomplicated virus infection.

The obvious response to influenza virus A under these circumstances was not remarkable. At no time was there observed the fever, anorexia, debility, or the respiratory syndrome associated with human influenza infection. However, there were certain characteristic serologic and hematologic changes which indicated that subthreshold non-symptomatic invasion by the virus did occur.

Hematologic.—Since leucocyte levels in monkeys vary widely and some individual animals exhibit marked fluctuations from time to time, each animal was repeatedly studied over a period of 1 to 3 weeks. Only those animals showing a relatively stable blood cell equilibrium were employed. The postinoculation data were evaluated in the light of the carefully established individual preinoculation base line equilibria.

Following instillation of the influenza virus, eight of the ten monkeys developed a significant leucopenia. While predominantly neutropenic in character, there was associated lymphopenia in seven of these animals during the period of maximum granulocyte depression. In three of the monkeys, the leucopenia developed as early as 24 hours after administration of the virus; in the others within 48 hours, except in one which showed a delayed response, first apparent on the 7th postinoculation day.

The initial cellular depression varied considerably in duration, being quite transitory, 1 to 2 days in a few animals and lasting for 10 days or more in others (see Fig. 1, monkey 5). The

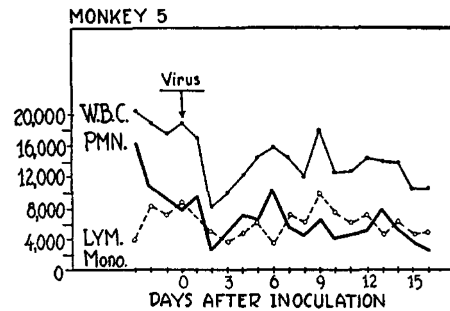

FIG. 1. Leucopenia following instillation of influenza virus; partial recovery of total white cells between the 6th and 9th day, followed by a secondary leukopenic phase.

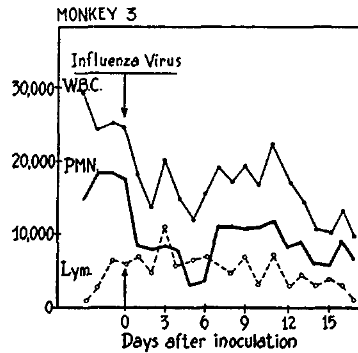

FIG. 2. Virus-induced leucopenia characterized by a primary sharp drop in granulocytes followed by a period of fluctuation of both granulocytes and lymphocytes.

average leucopenic depression persisted for from 3 to 7 days after virus instillation, and a reciprocal lymphocytosis was occasionally noted.

A secondary leucopenic relapse, following apparent recovery from the initial leucopenic phase, was observed in four animals (M 1, 3, 4, 6). In two of these monkeys (M 4, 6) this phenomenon appeared on the 12th postinoculation day and persisted for 4 to 5 days. A third animal (M 1) which showed a delayed initial leucopenia of 4 days' duration exhibited a brief return of granulocytes to normal levels 12 days after virus instillation, followed by a recurrent, asymptomatic leucopenia of 3 days' duration, which was similar to that seen in M 4 and M 6. Fig. 2 is illustrative of these fluctuations in the white blood cells as they occurred in monkey 3, with both granulocytes and lymphocytes participating.

The remaining two (M 1-6, 1-8) of the ten animals showed no significant peripheral blood cell responses to virus instillation. However, the appearance of virus-neutralizing antibodies in these animals was evidence that virus invasion did occur.

There were no qualitative or quantitative changes in the monocytes in any of the animals in these experiments. The red cells and hemoglobin were not significantly altered by the infection.

Serologic.—The immune responses of the twenty-four monkeys receiving influenza virus are tabulated in Fig. 3. Some of the animals received virus and streptococcus simultaneously, or

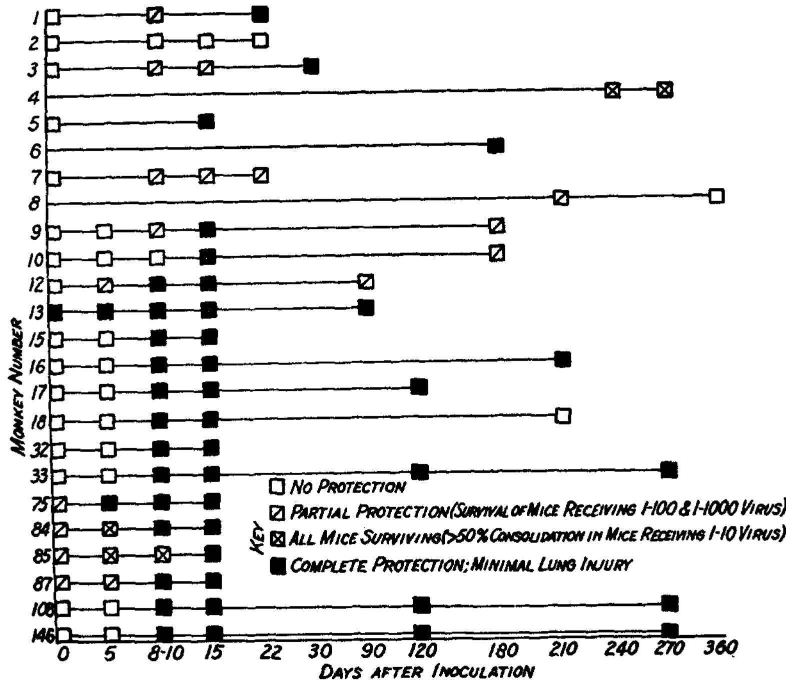

FIG. 3. Time of appearance, titer, and duration of influenzal antibodies (as determined by mouse protection test) in monkeys inoculated intranasally with influenza virus Type A. Each square represents the results of inoculations into nine mice of 0.05 cc. of mixtures containing 1 in 10 serum and 1 in 10, 1 in 100, 1 in 1000 dilutions of virus.

in sequence. These will be discussed in a subsequent separate communication. Since the virus antibody responses in our animals were apparently uninfluenced by bacterial agents, that aspect of the study can conveniently be included here.

Only five of the twenty-four monkeys included in our series possessed any demonstrable neutralizing antibodies prior to the experimental introduction of the influenza virus. Of the nineteen monkeys lacking neutralizing antibodies, fifteen were tested for this property 8 to 10 days after inoculation (Fig. 3), at which time thirteen had developed specific neutralizing antibodies, the serum from nine possessing antibodies in sufficient titer to afford complete protection to all mice. The serum of the other four monkeys (M 1, 3, 7, 9) in this group of thirteen offered partial protection, as a result of which mice receiving the 1 in 100 and 1 in 1000 dilutions of virus survived. Three of these four monkeys (M 1, 3, 9) showed complete mouse-protecting antibodies in their sera collected 21, 30, and 15 days respectively after inoculation.

As stated above, the sera of five of the twenty-four monkeys contained neutralizing antibodies prior to inoculation. An antibody titer sufficient to completely protect mice was present in one monkey (M 1-3). The remaining four animals (M 7-5, 8-4, 8-5, 8-7) had virus-neutralizing antibodies in low titer, which were subsequently increased 5 to 10 days after virus instillation.

The data as summarized in Fig. 3 show graphically that the antibodies reached a maximum titer between the 8th and 15th days after inoculation with influenza virus. At the end of 15 days the sera from sixteen of the nineteen monkeys which had shown no effective virus-neutralizing antibodies before inoculation, completely protected all mice against all virus dilutions.

To determine the duration of these virus-neutralizing antibodies, serum specimens were tested from thirteen monkeys 3 to 12 months after inoculation (Fig. 3). After 3 months, monkey 1-3 had sufficient antibodies to provide complete protection to mice, while the neutralizing capacity of the serum of monkey 1-2 protected mice against only the higher dilutions of virus.

Four months after infection, the sera of the four animals tested (M 1-7, 3-3, 1-08, 1-46) completely protected mice against all dilutions of virus. Three of these monkeys (M 3-3, 1-08, 1-46) still possessed sufficient antibodies 9 months after inoculation to afford complete mouse protection. At the end of 6 months after primary infection, the serum of M 6 neutralized all virus dilutions while the antibody titer in M 9 and 1-0 was somewhat diminished. Seven months after infection, M 1-6 still maintained completely protective antibodies; the serum of M 8 partially protected mice, but that of M 1-8 had lost its neutralizing antibodies. One year after inoculation, the serum of M 8 no longer contained any demonstrable neutralizing capacity. Eight and 9 months after inoculation of virus, M 4 possessed sufficient antibody to protect all the mice, although the mice receiving the serum and the highest concentration (1 in 10) of virus, when sacrificed, revealed an average of 45 per cent lung involvement.

In summary, specific immune antibodies were demonstrated in the serum in two of two monkeys, tested 3 months after infection; in all of four after 4 months, in all of three after 6 months, in two of three after 7 months, in the one animal examined after 8 months, and in all of the four after 9 months. The serum of the one monkey tested 12 months after primary infection failed to protect any mice.

Results of Reinoculations with Virus

The response to later reinoculation with the same influenza virus, administered as before, was studied in four originally non-immune monkeys. This afforded an opportunity to compare the initial response of monkeys lacking antibodies with that of the same animals possessing neutralizing antibodies from a previous inoculation with the same agent. Two of these animals (M 1-2, 1-3) received the virus 3 months after primary inoculation, while two (M 1-7 and M 6) were reinoculated 4 and 6 months later, respectively.

The reintroduction of the influenza virus did not produce any symptoms and thus differed in no way from the response following primary inoculation. The leucopenia characteristically appearing in these monkeys after receiving the virus for the first time, did not develop following reinoculation with the same agent.

Virus-neutralizing antibodies which had appeared after the first inoculation with virus were still present in the serum of all the monkeys in undiminished titer with the exception of M 1-2; in this case only sufficient antibodies were present to protect the mice receiving the higher dilutions of virus (1 in 100, 1 in

1000). Five days after reinoculation, the titer of this animal's serum had increased so as to confer complete protection on all mice.

Effects of Intratracheal Inoculation

In order to determine whether signs of influenza could be induced if the virus was introduced directly into the trachea, four monkeys were infected by this route.

All four animals were anesthetized with ether, as in the previous method, but only 1 cc. of virus suspension (3,000 mouse M.L.D.) was administered. In two of these monkeys (M 7-7, 7-8) the suspension was injected by No. 22 gauge hypodermic needle directly into the trachea. In the other two animals (M 9-0, 9-5) the suspension was injected after a small incision of the overlying tissues and exposure of the trachea. Following injection, the wound was closed with two No. 00 black silk sutures.

Both monkeys 7-7 and 7-8 on the 2nd day after introduction of the virus, exhibited signs suggestive of influenza as it occurs in man. Monkey 7-7 at this time appeared unusually quiet and inactive; its face was flushed and the conjunctivae were injected. Monkey 7-8 became quiet and listless; the face was flushed and there was marked lacrimation. Within the next 2 days both animals resumed normal appearance and activity. Hematologically, a sharp granulopenic leucopenia, with a reciprocal lymphocytosis was noted in both monkeys from the 3rd to 5th days after inoculation.

Monkeys 9-0 and 9-5 which received the virus following incision over the trachea, showed no obvious signs of infection. No leucopenia occurred in either animal and M 9-5, in fact, exhibited a transitory leucocytosis. It is possible that the surgical trauma incident to direct inoculation in these two animals was sufficient to neutralize the characteristic, virus-induced peripheral leucopenia.

Of the four monkeys in this study, one possessed complete virus-neutralizing antibodies before inoculation. The serum of the other three animals which had no protective properties before infection, developed complete mouse-protecting antibodies by the 14th day after virus administration.

In summary, of four monkeys receiving virus intratracheally, two displayed signs, after 48 hours, suggestive of influenza. Hematologically, a definite leucopenia was observed in the two animals which had not been subjected to surgical trauma. A characteristic serological response was observed in this group.

The Effect of Exposure to Cold on Resistance to Influenza Virus

Since the normal body temperature of the *rhesus* monkey is higher (101-103°F.) than that of the human being, a study was made to determine the effect of exposure to cold on infection with influenza virus (11).

Four of six monkeys (Fig. 4) which were kept in a room having a temperature between 4-6°C. were inoculated intranasally with influenza virus. All four animals showed a moderate response 24 to 48 hours after inoculation, with varying degrees of lethargy, weakness, anorexia, and respiratory distress. Three of the four monkeys died after 6, 11, and 17 days respectively, each exhibiting peribronchial areas of consolidation at necropsy. The specific virus was isolated from the lungs in one instance. The two control monkeys suffered no ill effects from the exposure. A distinct leucopenia (Fig. 4) involving both neutrophiles and lymphocytes

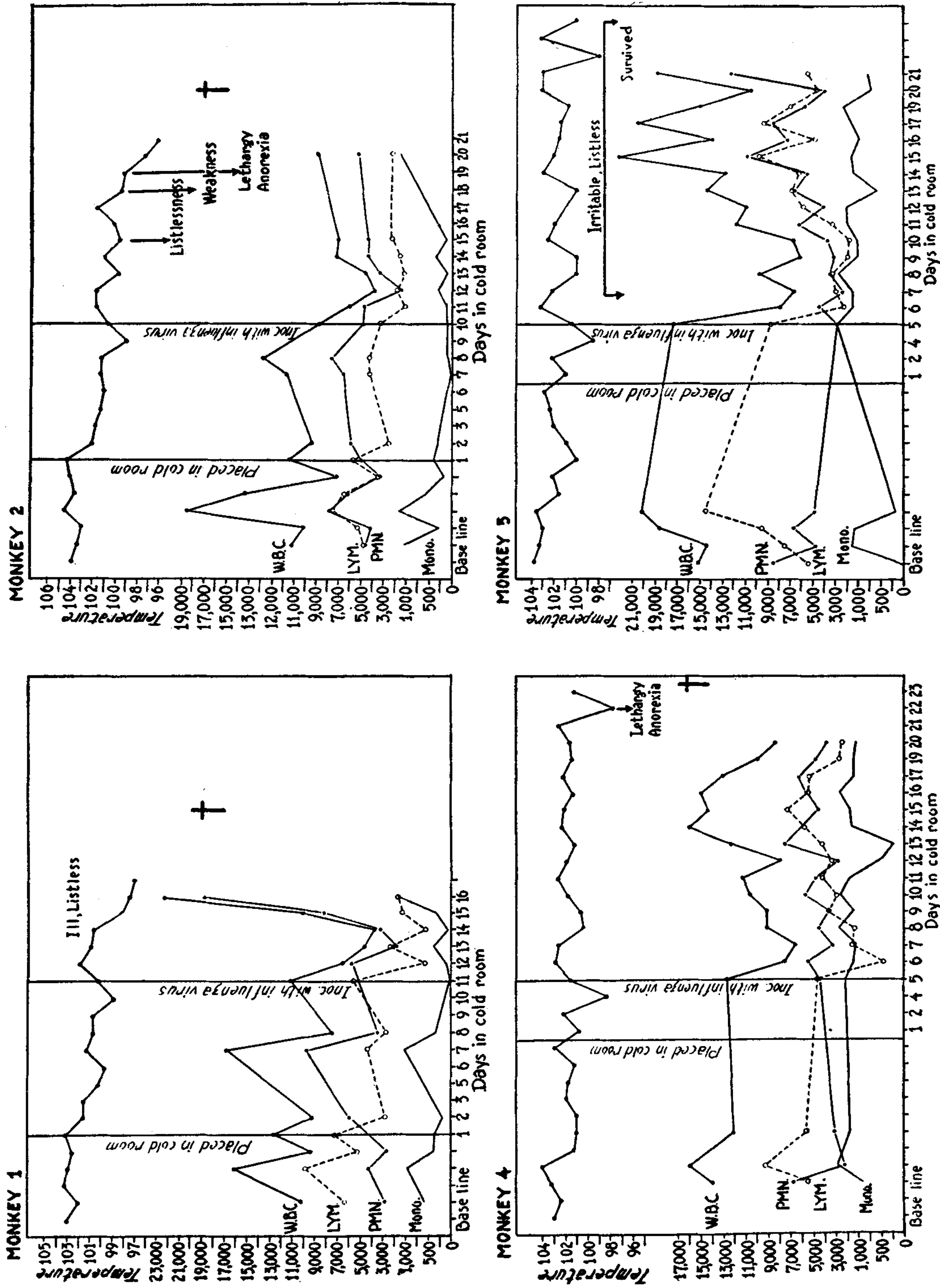

FIG. 4. The effect of lowered body temperature on the findings after experimental influenza infection.

developed in the four inoculated animals between the 1st and 5th days after instillation of the virus. Neutralizing antibodies were present in low titer on the 5th day after infection, and complete mouse-protecting antibodies were obtained on the 10th day.

Nutritional Deficiency and Susceptibility to Influenza Virus

Since healthy monkeys on optimum diets showed no obvious signs of infection with influenza virus A, the effect of nutritional deficiency was studied.

Seven monkeys which had been permitted to become nutritionally deficient on the synthetic diets described earlier (12), were inoculated intranasally with the virus of influenza. Of the seven animals so treated, five (M 2-7, 2-4, 6-9, 5-3, 5-6) died 2, 3, 7, 8, and 11 days, respectively, after inoculation. These monkeys showed marked anorexia after the administration of the virus and became progressively weaker and lethargic. At necropsy the lungs of four of the five monkeys (M 2-4, 4-7, 5-3, 5-6) showed small peribronchial areas of consolidation and the virus was recovered from the lung filtrates of three of these animals (M 2-4, 2-7, 5-6). The lungs of the fifth animal (M 6-9) showed diffuse atelectasis at necropsy.

Hematologically, the seven monkeys showed the leucopenia typical of this type of dietary deficiency (12) prior to inoculation with the virus. Following the instillation of the influenza virus, a further fall in white cells occurred promptly in four of the seven monkeys (M 2-4, 2-7, 6-9, 5-3), associated with a fulminant fatal course. The other three monkeys responded with an abortive transient leucocytosis. Neutralizing antibodies were found to be present in the serum by the 8th day after inoculation in the specimens obtained from the animals surviving this length of time (M 2-3, 5-1, 5-6 6-9). There was, thus, no difference in titer or time of appearance of antibodies between these monkeys and those on a normal diet.

The findings in monkey 5-3 (Fig. 5) are typical of the effect of the virus in a leucopenic, nutritionally deficient monkey, which had responded classically to folic acid earlier. Following inoculation the total white count fell from 3700 to 2350 in 24 hours with a concomitant drop in neutrophiles from 2146 to 1363. The superimposed leucopenia persisted for 72 hours at which time the total white count was 2200 with 1700 neutrophiles. On the 4th and 5th days the number of white cells returned to the preinoculation, but still leucopenic, level, varying between 3750 and 3550, but on the 6th day there was a secondary depression with the total white elements falling to 1350 with only 945 neutrophiles and 405 lymphocytes. The monkey died on the 8th day. Virus-neutralizing antibodies were present in the serum,—drawn before death on the 8th day,—in sufficient titer to protect mice against the 1 in 100 and 1 in 1000 dilutions of virus. At necropsy the upper one-fifth of the right lower lobe, including the upper and lateral visceral pleural surfaces, was dark red and firm. One small dark red patch 0.5 cm. in diameter was noted in the lower left lobe. The rest of the lower right lobe was studded with small peribronchial areas of consolidation.

In summary, five of seven nutritionally deficient monkeys with preinfection leucopenia, died within 2–11 days following inoculation with influenza virus. A further specific leucopenia was observed in four of the five animals. The specific virus was isolated from the lungs of three of the five monkeys which died. In the gross, the lungs of four of the five monkeys which died showed peribronchial consolidation, while those of the fifth animal displayed diffuse atelectasis. The results obtained in this nutritionally deficient group were in sharp contrast with those in normal monkeys described earlier in this paper, in which no obvious signs of the virus infection and no fatalities occurred. The

immunologic responses were essentially the same in both groups. However, the leucopenia in the latter group was more profound and specific, owing to the nutritional leucopenia prior to virus infection.

Results of Inoculations with Inactivated Virus

Since no symptoms had been observed following the administration of living virus, the presence of virus-neutralizing antibodies was employed as a serological

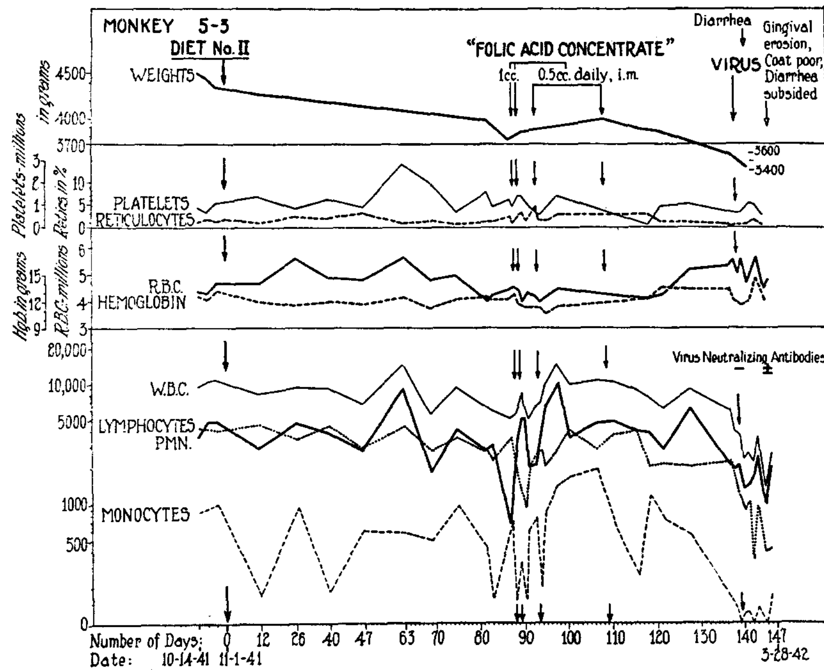

FIG. 5. Influence of a deficiency diet on influenza infection. After 155 days on diet 2 a definite leucopenia developed. Intranasal inoculation with influenza virus at this time resulted in a more profound leukopenia and death on the 8th day of the infection. Note effect of folic acid on granulocytes earlier in experiment.

criterion of invasion. We wished therefore to determine whether *inactivated* virus administered intranasally would induce the production of virus-neutralizing antibodies.

Two groups of monkeys (Fig. 6) were inoculated intranasally with inactive virus. Group I (M 6-2, 6-3, 6-5) consisted of monkeys whose sera showed a low titer of antiinfluenza immune bodies originally. Group II (M 6-4, 7-9, 8-1, 8-2, 8-3) included monkeys possessing no demonstrable virus-neutralizing antibodies in their sera, as determined in tests preliminary to inoculation.

In Group I there was a marked increase in antibody titer 10 to 15 days after inoculation,

which conferred complete protection with minimal lung pathology in all mice tested. In Group II the appearance of neutralizing antibodies was detected 10 days after instillation of inactivated virus, at which time the serum of four of the five monkeys protected all mice receiving the higher dilutions of virus (1 in 100 and 1 in 1000). Fifteen days after inoculation, all serum samples of this group showed a further increase in titer of the neutralizing antibodies. None of the monkeys in either of these two groups exhibited significant peripheral blood cell changes following instillation of the inactivated virus.

On the basis of these findings, in which antibodies specific for the influenza virus became demonstrable or were increased in titer significantly, it was concluded that heat-inactivated virus is antigenic when introduced intranasally in monkeys. The duration of the immunity, however, was not determined.

Effects of Inactive Virus Followed by Active Virus

Four of the monkeys in this series (M 6-2, 6-3, 6-4, 6-5) received living influenza virus 14 to 19 days after the instillation of the inactivated virus. None

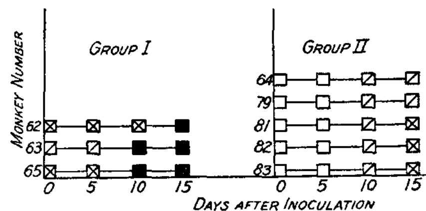

FIG. 6. Time of appearance and titer of influenzal antibodies in monkeys inoculated intranasally with inactivated virus ("booster effect"). Group I with preinoculation low titer antibodies; Group II with no previous virus-neutralizing antibodies. Each square represents the results of inoculations into nine mice of 0.05 cc. of mixtures containing 1 in 10 serum and 1 in 10, 1 in 100, 1 in 1000 dilutions of virus.

of the animals became ill, but three of them developed leucopenia. In one animal (M 6-2) a neutropenia with reciprocal lymphocytosis appeared within 24 hours after the virus was administered, persisting until the 4th day.

Results of Inoculation with Buffered Saline

Inasmuch as the influenza virus had been suspended in sterile buffered saline in the preceding experiments, a control study was made to determine the possible influence of this agent, *per se*, when administered under ether anesthesia. Four normal monkeys were inoculated intranasally with 3 cc. of sterile saline 17 days before the subsequent administration of inactivated virus. No blood cellular or humoral changes were observed.

RÉSUMÉ AND DISCUSSION

The foregoing experimental data indicate that normal *rhesus* monkeys prove resistant on intranasal inoculation with the PR8 strain of influenza virus A. Following the introduction of the virus the animals remained normal to all ap-

pearances and none of the characteristics which typify influenza in man and in the smaller experimental animals ordinarily employed in such studies, were observed.

However, in response to virus intranasally administered, the monkeys presented characteristically a granulocytopenia between the 1st and 7th postinoculation days, followed by spontaneous recovery, or in some instances by a secondary leucopenia. Virus-neutralizing antibodies became demonstrable in the serum of most of the monkeys about the 8th day, approximately at the time of recovery from the leucopenia. Thus following infection there are changes in the body equilibrium, characterized by an altered blood picture and a specific antibody response, which may be related to the defense mechanism.

Nutritionally normal monkeys reinoculated with living virus, while possessing a high titer of circulating neutralizing antibodies, did not develop the leucopenia observed after primary inoculation, thus supporting the suggestion of an interrelationship between the cellular and humoral phenomena. These observations, however, do not permit the assumption that the virus-neutralizing antibodies and hematologic reactions constitute the sole factors determining the resistant or susceptible state. The presence or absence of specific antibodies at the time of influenza virus inoculation did not effect materially the obvious manifestations of infection.

In contrast to the apparently resistant state of normal monkeys under optimal conditions to intranasal inoculation with influenza virus A, the animals subjected to experimentally altered conditions of diet, exposure, or route of inoculation, showed increased susceptibility. Whereas ten normal monkeys exhibited no obvious effects following virus instillation, five of seven nutritionally deficient monkeys succumbed to this infectious agent, and the four animals exposed to lowered temperatures displayed signs consistent with influenza, with fatalities in three of the four so treated. When the virus was introduced intratracheally, two of four monkeys became ill on the 2nd day, but recovered spontaneously. Although there were these obvious differences, the immunologic responses were essentially the same. Monkeys receiving heat-inactivated virus formed immune bodies, without alteration in the hematologic pattern. In consequence, it can be assumed that the antibody response in all of the animals was dependent only upon the introduction of an antigen, living or dead, but the relative signs of resistance or susceptibility to influenza virus of these monkeys did not appear to depend directly upon this humoral response.

The leucopenia, which characteristically followed the primary introduction of living virus, was noticeably absent in immune monkeys reinoculated with influenza virus and in those receiving heat-inactivated virus. Thus, the leucopenia was indicative of primary invasion with influenza virus, but its possible rôle in defense is difficult to assess in the absence of superimposed pyogenic infection, and without even transitory symptoms. The factor or factors, therefore, related to the relative resistance to influenza virus of the monkey (*Macaca*

mulatta) under optimal nutritional conditions cannot be attributed solely to the cellular and/or humoral responses.

The natural outer defense barriers of intact epithelium could possibly be one of the factors responsible for the absence of symptoms in normal monkeys following intranasal instillation of the virus. The introduction of virus directly into the trachea by-passes the primary barriers afforded by the upper respiratory epithelium, and probably results in a quantitatively greater invasion. The fact that two of four monkeys inoculated intratracheally displayed some signs compatible with influenza, would suggest that this hypothesis is tenable. The relative resistance of normal tracheobronchial epithelium may have accounted for the lack of obvious signs in the other two monkeys. Burnet (9) in a study of influenza in *cynomolgus* monkeys was able to induce influenza only by the intratracheal route. On the basis of our findings and his we are inclined to agree with him that the tracheobronchial epithelium is an important factor governing the responses of the monkey to the virus; and that under normal conditions the more vulnerable bronchiolar epithelium is not reached.

The relation of nutritional deficiency to increased susceptibility to infection is a well known clinical observation, and spontaneously occurring, and experimentally induced infections in states of nutritional deficiency have been described by us and by others (13–20). In the observations noted here, the rôle of optimum nutrition in resistance is apparent. The antibody response in nutritional deficiency did not differ from that observed in normal monkeys; but the leucopenia which occurred on a nutritional basis was still further accentuated following virus inoculation. It may well be responsible for the development of secondary pyogenic infections (13). A part of the deficient monkey's increased susceptibility may be due to a greater vulnerability of the affected epithelial barriers.

The differences in susceptibility between the normal monkeys on the one hand and the nutritionally deficient animals and those inoculated intratracheally on the other, may therefore be based on a qualitative difference in respiratory epithelial and other tissue barriers, with a resulting quantitative difference in actual virus invasion, followed by a more profound granulopenic phase and potential pyogenic complications.

The rôle of exposure and cold in upper respiratory infections also confirms earlier observations. Pasteur (21) in the early days of bacteriology attributed the resistance of chickens to the anthrax bacillus as due to the high body temperature of the fowl. On immersing the bird's feet in cold water, and thereby lowering the body temperature, fatal infection occurred. The increased susceptibility to influenza virus, of monkeys subjected to lowered temperatures, may be attributed to the lowering of the normal body temperature to a level more closely approximating the optimal temperature range in which the influenza virus is effective; *i.e.*, to the temperature of man, a susceptible species. The chilling effect, *per se*, may also be a contributing factor.

CONCLUSIONS

1. *Macaca mulatta* monkeys on a normal diet have proved resistant to intranasal but not to intratracheal inoculation of influenza virus.

2. Neutralizing antibodies appeared 8 to 10 days after inoculation with either living or heat-inactivated virus. The antibodies were noted to be still present as long as 9 months after infection with living virus.

3. A specific granulopenic leucopenia characteristically followed primary influenza virus inoculation, regardless of altered conditions of diet, exposure, and route of inoculation, but it was not observed in monkeys previously infected with the same virus, all of which invariably survived.

4. Nutritional deficiency and exposure to cold increased the susceptibility of monkeys on intranasal instillation of the virus; the leucopenia was profound and fatalities frequently occurred even though neutralizing humoral antibodies developed as promptly and in relatively the same titer as under optimum nutritional conditions.

BIBLIOGRAPHY

1. Smith, W., Andrewes, C. H., and Laidlaw, P. P., *Lancet*, 1933, **2,** 66.
2. Andrewes, C. H., Laidlaw, P. P., and Smith, W., *Lancet*, 1934, **2,** 859.
3. Francis, T., Jr., *Science*, 1934, **80,** 457.
4. Stuart-Harris, C. H., *Brit. J. Exp. Path.*, 1936, **17,** 324.
5. Stuart-Harris, C. H., *Brit. J. Exp. Path.*, 1937, **18,** 485.
6. Shope, H. E., and Francis, T., Jr., *J. Exp. Med.* 1936, **64,** 791.
7. McIntosh, J., and Selbie, E. R., *Brit. J. Exp. Path.*, 1937, **18,** 334.
8. Vieuchange, M. J., *Bull. Acad. med.*, Paris, 1939, **121,** 100.
9. Burnet, F. M., *Australian J. Exp. Biol. and Med. Sc.*, 1941, **19,** 281.
10. Woolpert, O. C., Schwab, J. L., Saslaw, S., Merino, C., and Doan, C. A., *Proc. Soc. Exp. Biol. and Med.*, 1941, **48,** 558.
11. Saslaw, S., and Hudson, N. P., unpublished data.
12. Wilson, H. E., Doan, C. A., Saslaw, S., and Schwab, J. L., *Proc. Soc. Exp. Biol. and Med.* 1942, **50,** 341.
13. Saslaw, S., Schwab, J. L., Woolpert, O. C., and Wilson, H. E., *Proc. Soc. Exp. Biol. and Med.* 1942, **51,** 391.
14. Saslaw, S., Doctoral Dissertation, Columbus, The Ohio State University, 1942, 51.
15. Langston, W. C., Darby, W. J., Shukers, C. F., and Day, P. L., *J. Exp. Med.*, 1938, **68,** 923.
16. Janota, M., and Dack, G. M., *J. Infect. Dis.*, 1939, **65,** 217.
17. Topping, N. H., and Fraser, H. F., *Pub. Health Rep., U. S. P H. S.*, 1939, **54,** 416.
18. Tomlinson, T. H., Jr., *Pub. Health Rep., U. S. P. H. S.*, 1939, **54,** 431.
19. Day, P. L., Langston, W. C., Darby, W. J., Wohlin, J. G., and Mims, V., *J. Exp. Med.* 1940, **72,** 463.
20. Chapman, O. D., and Harris, A. E., *J. Infect. Dis.*, 1941, **69,** 7.
21. Pasteur, L., Joubert, J. F., and Chamberland, C., *Bull. Acad. méd.*, Paris, 1878, series **2, 7,** 432.