JOURNAL OF HYGIENE, EPIDEMIOLOGY, MIKROBIOLOGY
AND IMMUNOLOGY 1958 — II — 9

AN ATTEMPT TO TRANSMIT THE HUMAN INFLUENZA VIRUS STRAIN A-SING 57 TO SWINE (PRELIMINARY REPORT)

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In the experiments of the first group of workers on the biology of the influenza virus up to 1948 [Mote[1])], three species of animals were held to be regularly susceptible to the human influenza virus (usually type A): ferrets as the most susceptible, white mice upon adaptation, and also swine to some strains (WS, PR8). Mote and Jones, however, later demonstrated that the susceptibility of these animals to human strains is not constant.

The subsequent discovery of susceptibility to the human influenza virus in the porcupine [Stuart-Harris[2])], the Chinese mink [Tang[3])], in certain species of squirrels, and, under special conditions in guinea pigs [Dujaric de la Rivière[4]), Patočka[5])] has no significance in the study of the pathogenesis of infection with this virus.

Inspired by one theory on the genesis of the swine influenza virus and by an unsupported report of an influenza epidemic among hogs observed in China previous to a human epidemic caused by the virus A-Sing 57, we thought it interesting to determine to what extent swine are susceptible to the virus A-Sing 57 under conditions which, though very similar, nevertheless, differed from those in the experiments of Shope and Francis[6]). The main difference lay in the modification of the technique employed in detecting the influenza virus and demonstrating specific antibodies.

As far as we know from accessible literature, the first successful experimental transmission of virus WS to swine was performed by Elkeles[8]) in 1934. Later, in 1936, Shope and Francis infected 6—14-week-old pigs with a 10% suspension of mouse lung tissue infected with strain WS, and with the then recently isolated PR8. The inoculation was made intranasally under light ether narcosis. Signs of the disease, when the virus alone was used, were very mild and not constant, and in most cases hardly recognizable. Swine with symptoms had only a low grade fever the day following inoculation. At autopsy the animals showed scattered lobular atelectasis in the lungs from which the virus was recovered. Lung tissue free of lesions was free of the virus.

When in another series a mixture of influenza virus and *Haemophilus influenzae suis* was inoculated, a clinically apparent illness was produced with

Table 1. First Swine Passage.

Pig No.	Isolation / Day-after inf.	2.	4.	7.	23.	Antigen / Day-after inf.	HI — Antibodies: Before inf.	13.	20.	46.
223	Virus	—	—	—		A-Sing 57	32—	32—	128+	
	H. infl. suis	+	+			Sw.			32—	
224	Virus	+	—	—	—	A-Sing 57	32—	32—	128+	
	H. infl. suis	+	+		+	Sw.			32—	
225	Virus	+	+	+	—	A-Sing 57	32—	512+	512+	256+
	H. infl. suis	+	+		+	Sw.			32—	

Table 2. Second Swine Passage.

Pig No.	Isolation / Day-after inf.	Before inf.	4.	7.	11.	24.	Antigen / Day-after inf.	HI — Antibodies: Before inf.	14.	24.
226	Virus		+	—	—		A-Sing 57	32—	64+	64+
	H. infl. suis	—	—		—	—	Sw.			32—
227	Virus		+	—	—		A-Sing 57	32—	64+	64+
	H. infl. suis	—	—		—	—	Sw.			32—

Table 3. Third Swine Passage.

Pig No.	Isolation / Day-after inf.	Before inf.	4.	7.	11.		Antigen / Day-after inf.	HI — Antibodies: Before inf.	14.	
232	Virus		+	Hitherto not performed			A-Sing 57	32—	32—	
	H. infl. suis	—	+	+	+		Sw.	32—		
233	Virus		+	Hitherto not performed			A-Sing 57	32—	32—	
	H. infl. suis	—	+	+	—		Sw.	32—		

temperatures usually lasting not more than three days. The clinical picture was qualitatively similar to the one produced by the swine influenza virus, differing only in the quantitative aspect. Neutralizing antibodies and immunity to the homologous virus were demonstrated in infected animals. The human virus passaged on swine neither underwent any changes in its properties nor was its virulence for swine enhanced. Mote and Fothergill[1]) in 1939 confirmed previous findings with the addition that human strains of *H. influenzae* did not have a synergistic influence on viral infection.

In connexion with the above it is well to bear in mind Shopes's[9]) report (1939). He demonstrated neutralizing antibodies in older pigs in New Jersey

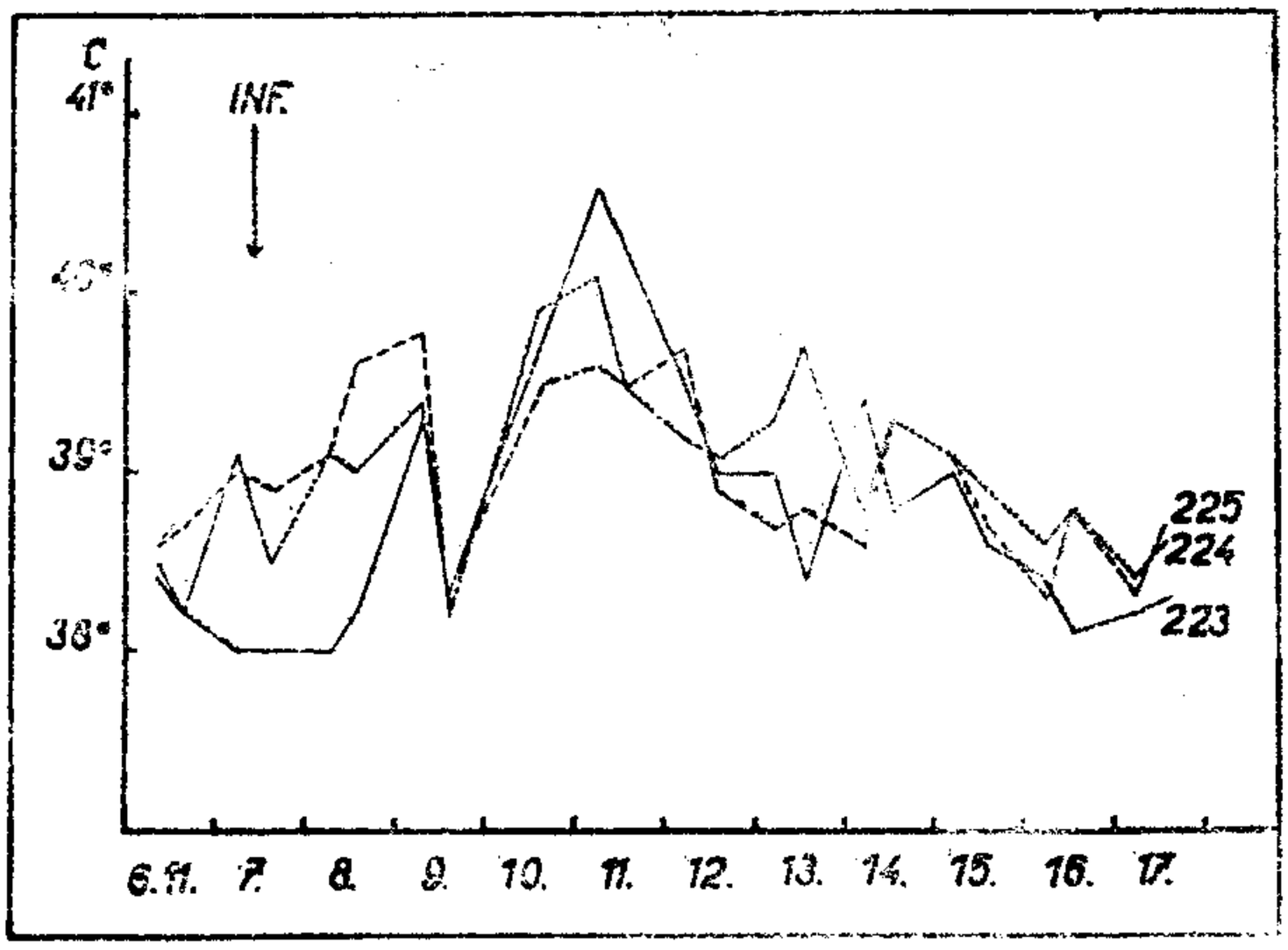

Fig. 1. Temperature curve of pigs in the first passage measured twice daily at 8 and 13 o'clock.

which were apparently infected during a human epidemic; pigs born after the epidemic had no demonstrable antibodies.

EXPERIMENTAL

In the first series of our experiments the A-Sing 57 strain was used which was furnished by Dr. Tůmová of the Institute of Epidemiology and Microbiology. We were not able to find out how many egg passages the virus had undergone. On routine examination we found the haemagglutination titre to be 1280 + with ½% chick blood and 640 + with guinea pig cells.

Swine were inoculated intranasally (hogs No. 223, 224, 225) with 2 cc. of allantoic fluid under light ether narcosis on first passage.

On second passage pig No. 226 was inoculated also under light narcosis with 3 cc. of suspension of virus recovered from the previous swine passage after three amniotic and one allantoic passages. Since the haemagglutination titre of this passage was very low (1:40 +, 1:80 ±), the virus was concentrated by adsorption on red blood cells with subsequent elution to a titre of 1:640. Pig No. 227 of the same passage was infected with 4 cc. of amniotic fluid from embryos, inoculated with the virus recovered from the first swine passage, but without any attempt to concentrate it (the third amniotic passage had an haemagglutination titre of 1:80).

On third swine passage the virus recovered from the second swine passage was used. Two animals (pigs No. 232, 233) were inoculated with 3 cc. of allantoic fluid with an haemagglutination titre of 160—, 320— (after three amniotic and one allantoic passages).

Animals used in the first passage were young pigs weighing around 15—16 kg; *H. influenzae suis* was recovered from the nasal mucous membranes of all three animals. Animals used in the second passage were older, weighing around 25—28 kg.; in these *H. influenzae suis* was not demonstrated. For the third passage young pigs, weighing around 12—15 kg. were selected; from these the haemophilus was not demonstrable prior to infection with A-Sing 57, but following inoculation it was repeatedly recovered during the experiment.

Samples for virus cultivation were made with cotton wool swabs on extra long wires so as to enable the taking of material from the entire length of the turbinates. The swabs were then soaked in Hanks' solution, after centrifugation penicillin in the amount of 1,000 units and streptomycin in the amount of 1,000 gamma per cc. of centrifuged eluate were added. This preparation was then inoculated into amniotic sacs of eleven-day chick embryos.

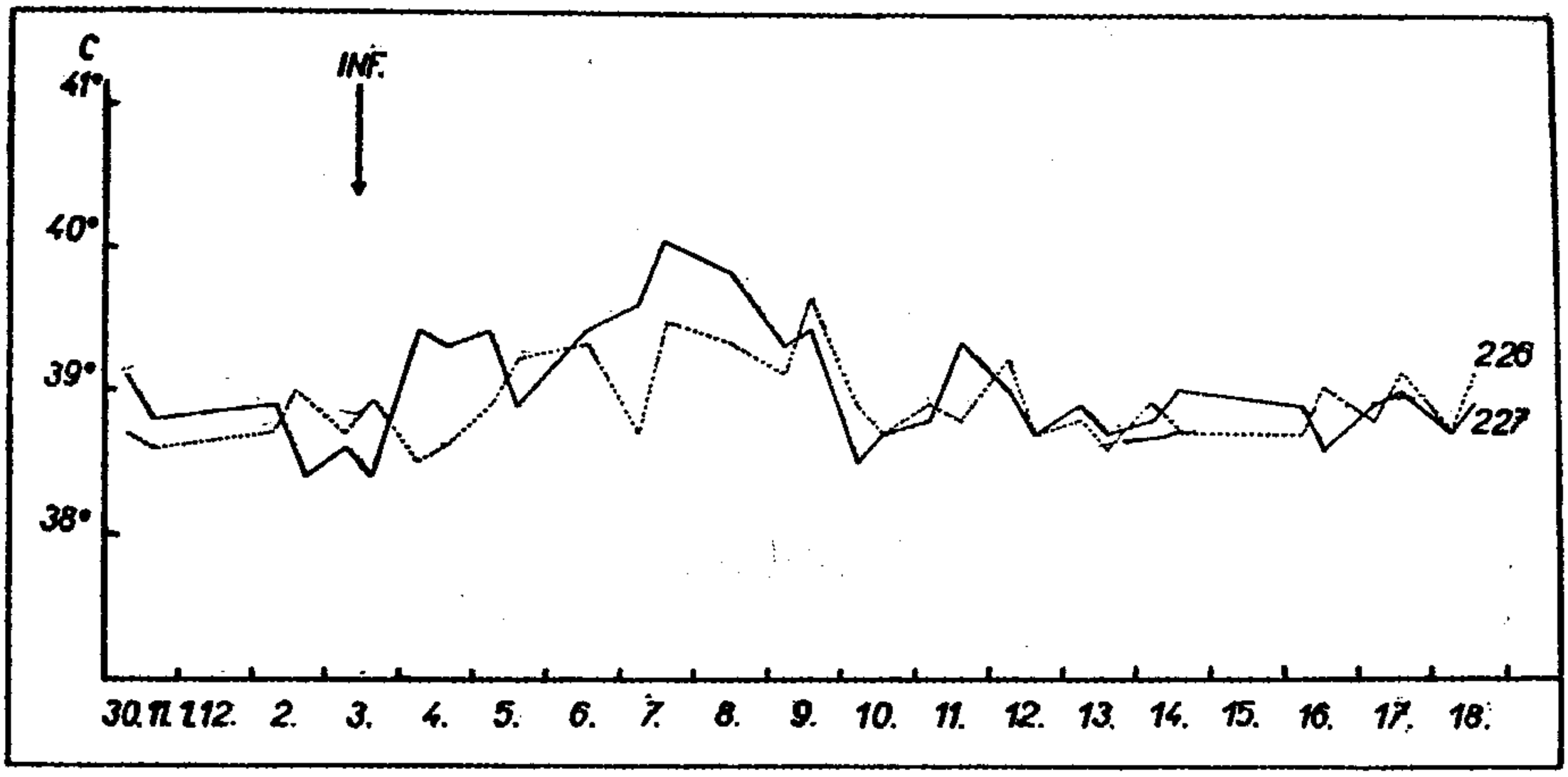

Fig. 2. Temperature curve of pigs in the second passage measured twice daily (8 and 13 o'clock).

Blood for examination was withdrawn from the animals by intracardiac puncture. Temperature was taken per rectum.

Haemagglutination-inhibition test was made according to Salk, employing ½% suspension of chick red blood cells (in dilution from 1:32). Strains recovered from nasal swabs were tested with rat and hamster anti-A-Sing 57, -A'-Most, -B. Bratislava, -C, -D, and anti-swine-influenza immune sera.

RESULTS

Results testifying to the persistence, and, in our opinion, also the propagation of the virus in the mucous membranes of the upper respiratory tracts in swine, are given in three charts, one for each passage. The charts also reveal a gradual rise of the haemagglutination-inhibiting antibody titre against the employed strain, which proves that all the animals developed an illness caused by this virus. (Tab. 1, 2, 3.)

Clinical signs, i. e., a rise in temperature, are given for each group in Fig. 1, 2, 3.

DISCUSSION AND CONCLUSIONS

It is evident from the results of our experiments that young pigs are susceptible to virus A-Sing 57, although the strain must have undergone a series of egg passages previous to employment by us, which, as is well known, diminishes the infectiousness of the influenza virus for man

and ferrets to a minimum. The differences in recovery of the virus and of the titre of antibodies, as given in Tab. 1 and 2, point to considerable susceptibility of young pigs. We do not disregard the fact that the presence of the autochthonic *H. influenzae suis* plays a role of synergistic bacterial agent. The susceptibility of the animals is not the same, as is evident in Tab. 1.

The clinical reaction to infection is best demonstrated on temperature curves. In the first two passages the apex is reached on the fourth day; at the same time, average values in young animals are higher. As against the experiments of Shope and Francis[6]), it seems (cum grano, since the third series of our experiments has not yet been terminated), that the virulence of the virus on adaptation to swine is to certain degree enhanced, taking into consideration the temperature curves of the third swine passage (Fig. 3), where the highest temperature is reached already on the second day.

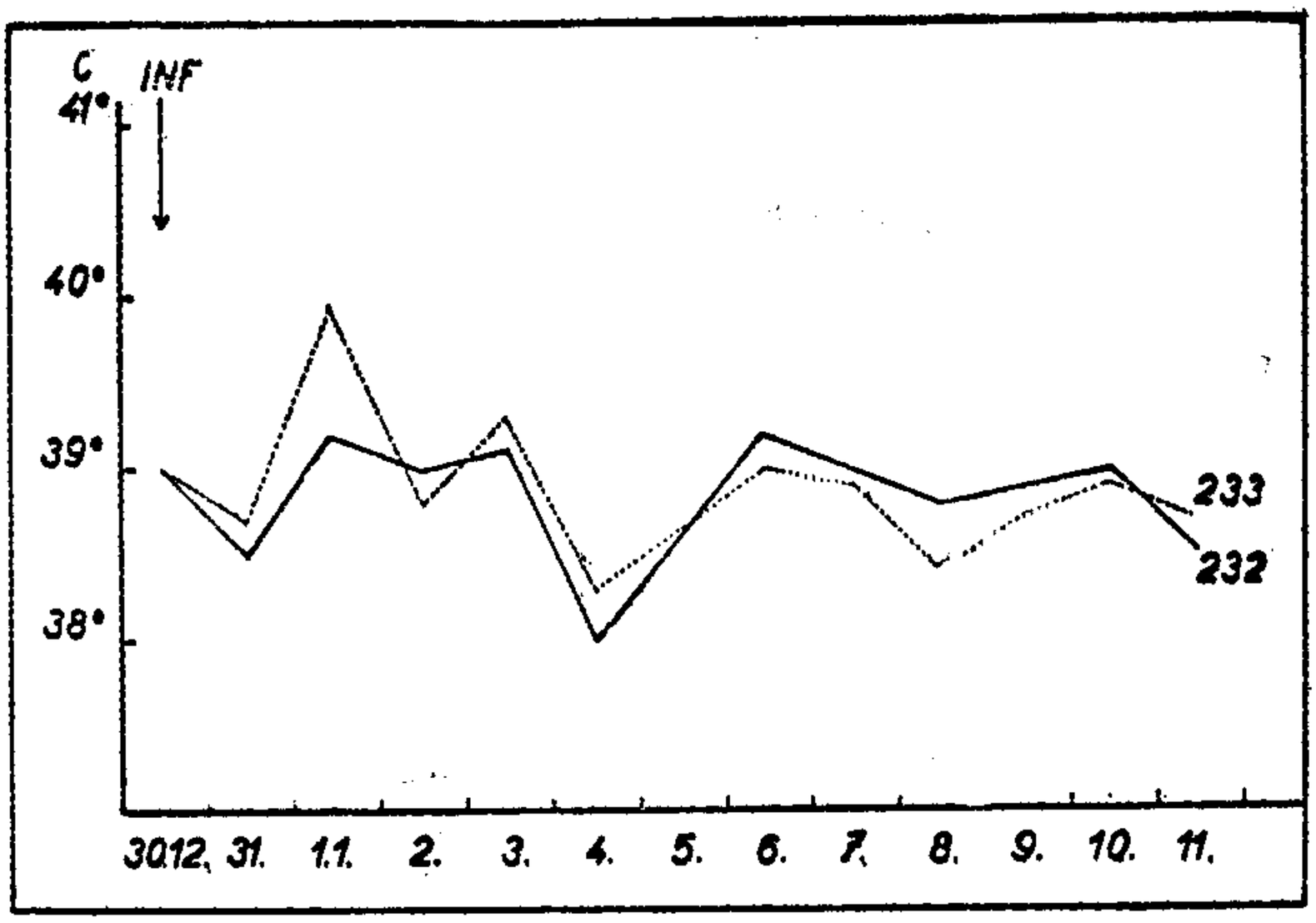

Fig. 3. Temperature curve of pigs in the third passage measured once daily.

The unsuccessful recovery of the virus from pig No. 223, and also from No. 224, may be ascribed to the then not very exact technique.

Strains recovered from swine (best seen in the first passage) seem to behave like strains newly isolated from human disease, for, after infection with virus however concentrated, they have on isolation (which is not after all very difficult) a low haemagglutination titre, only relatively slowly enhanced on subsequent egg passages.

The adapted virus has a capacity to agglutinate porcine red blood cells. This finding is subject to further study.

According to only one hitherto performed experiment, is does not seem that the virus in the second swine passage was transmittable by contact to an healthy animal placed in the same pen.

On the whole, our experiments coincide with those of Elkeles, Shope and Francis, performed more than 20 years ago, since they confirm and extend the findings of the above authors to the newly found human virus type A, designated as A-Sing 57.

We are also of the opinion, that our findings do not, for the time being, warrant us to draw any far reaching conclusions.

RÉSUMÉ

Il resort des résultats que les jeunes cochons sont demonstrativement susceptible à l'infection par le virus A-Sing 1/57 aussi qu'il était passagé plusieurs fois l'embryon de poulet par quoi, comme il est connu génerallement, l'infectiosité du virus grippal pour l'organisme humain aussi bien que pour le furet est réduite au minimum. Comme il résulte de la différence des isolations du virus et du titre des anticorps entre le premier et le deuxième tableau, les petits cochons sont beaucoup plus sensible. Nous ne contestons pas que la présence de Haemophilus influenzae suis

autochthon joue un rôle d'un agent bactériel agissant synergiquement. La susceptibilité des animaux n'est pas égale, comme on peut bien voir du premier tableau.

La réponse clinique de l'infection est démonstré le mieux par des courbes ajoutées de la températures. Chez les premières deux passages, la température culmine le quatrième jour, les valeurs moyennes chez les petits animaux étant plus hautes. À la différence des expériences de Shope et Francis il semble (avec une certaine réserve, car les expériences dans la troisième passage ne sont pas cependant finies) que la virulence du virus s'élève un peu par l'adaptation à cochon, ce qu'il résulte de la courbe des températures de la troisième passage par les cochons (tableau No, 3), où on trouve le maximum d'élévation de la température déjà pendant le deuxième jour.

Le fait qu'on n'a pas réussi à isoler le virus chez le cochon No 223, et dans certaine mesume aussi chez le cochon No 224, qui d'après l'élévement du titre des anticorps ont évidemment fait l'infection, peut être expliqué par des méthodes encore imparfaites de ce temps-là.

Les souches isolées des cochons (qu'on peut voir le mieux chez la première passage) paraissent se conduire comme des souches fraîchement isolées de l'infection humaine, ayant independent de la concentration du virus utilisé pour l'infection expérimentale à l'isolation (qui est l'ailleurs comparativement facile) un haut titre de haemagglutination qui ne s'élève pendant des passages ultérieures sur des oeufs que rélativement lentement.

D'une seule expérience faite jusqu'ici, il ne semble pas que le virus de la deuxième passage sur des cochons par un contact aux ultérieurs soit transmissible animaux sains placés dans la même cage.

Généralement, nos expériences s'accordent avec celles-ci exécutées par Elkeles, Shope et Francis, il y a 20 ans, car elles confirment et amplifient (en usant des méthodes modernes de travail) des indications de ces auteurs aussi pour la virus humain du type A récemment reconnu, désigné comme A-Sing 1/57.

Nous constatons aussi que nos résultats ne nous autorisent provisoirement aux nulles conclusions épidémiologiques persuasives.

ZUSAMMENFASSUNG

Wie es sich aus den Ergebnissen herausstellt, sind die jungen Schweine nachweislich zur Infektion mit dem Virus A-Sing 1/57 trotzdem empfänglich, dass dieses sicher eine ganze Reihe von Passagen auf Hühnerembryos durchgemacht hat, was — wie allgemein bekannt — die Infektiosität des Influenzavirus für den menschlichen Makroorganismus so wie für das Frettchen aufs Minimum erniedrigt. Wie es aus dem Unterschied in den Isolationsversuchen und im Titer der Antikörper zwischen der ersten und der zweiten Tabelle sichtbar ist, sind die kleinen Ferkel deutlich empfindlicher. Wir schliessen nicht aus, dass die Anwesendheit des autochthonen Haemophilus influenzae suis die Rolle eines synergisch wirksamen bakteriellen Faktors spielt. Die Empfänglichkeit der Tiere ist nicht gleich, wie man besonders gut aus der ersten Tabelle feststellen kann.

Die klinische Antwort ist am besten aus den beigefügten Temperaturkurven merklich. Die Temperatur kulminiert bei den ersten zwei Passagen am vierten Tag, wobei die durchschnittlichen Werte bei kleinen Tieren noch höher sind. Im Unterschiede zu den Versuchen von Shope und Francis scheint (mit einer bestimmten Reserve, weil die Versuche in der dritten Passage bisher nicht abgeschlossen wurden) die Virulenz des Virus durch dessen Adaptation auf das Schwein gewissermassen sich zur erhöhen. Dies erschliessen wir aus der Temperaturkurve der dritten Schweinepassage (Tabelle No 3), wo es zum Maximum der Temperaturerhöhung schon am zweiten Tag kommt.

Negative Ergebnisse der Isolationsversuche beim Schwein No 223, und zum gewissen Masse auch beim Schwein No 224, welche nachdem erhöhten Titer der Antikörper offensichtlich die Infektion durchgemacht haben, könnten vielleicht teilweise der damals noch unvollkommenen Arbeitsmethode zugeschrieben werden.

Die aus Schweinen isolierten Stämme (was am besten bei der ersten Passage sichtbar ist) scheinen sich so zu verhalten wie die frisch aus einer Menscheninfektion isolierten Stämme, denn sie haben unabhängig von der Konzentration des zur Experimentalinfektion verwendeten Virus bei der Isolation (die übrigens im Allgemeinen leicht ist) einen niedrigen Hämagglutinationstiter, der im Verlauf weiterer Eierpassagen nur Verhältnismässig langsam aufsteigt.

Aus dem einzigen bisher verwirklichten Versuch scheint das Virus aus der zweiten Schweinepassage nicht durch Kontakt übertragbar zu sein auf ein weiteres gesundes Tier, das in denselben Käfig gegeben wurde.

Im Ganzen stimmen unsere Versuche sehr mit den vor mehr als 20 Jahren von Elkeles, Shope und Francis durchgeführten Versuchen, weil sie (unter Benützung einer neuzeitlichen Arbeitsmethode) die Angaben der erwähnten Autoren auch für das neu erkannte Virus, Typ A, bestätigt, das man als A-Sing 1/57 b zeichnet.

Wir stellen auch fest, dass uns unsere Ergebnisse bis auf Weiteres zu keinen überzeugenden epidemiologischen Schlussfolgerungen berechtigen.

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Received January 14, 1958.

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