

# HEMATOLOGICAL CHANGES FOLLOWING INFECTION OF ANIMALS WITH THE VIRUS OF EPIDEMIC INFLUENZA

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Of the animals susceptible to the virus of epidemic influenza, ferrets, piglets, and white mice are of the greatest practical value. Ferrets may very readily be infected by the nasal secretions of an influenza patient, whereas white mice are less susceptible to infection by this means; piglets only become susceptible to the virus of epidemic influenza after it has been passed through ferrets or white mice.

We know of no papers dealing specifically with hematological changes taking place in ferrets, piglets, and white mice following infection with influenza virus. R. Shope's paper (1931), dealing with the etiology of swine influenza, presents hematological data for swine before and after infection with a mixture of filtrable virus and Hemophilus influenzae.

In our present paper we present the results of an investigation into the hematological changes occurring in ferrets, white mice, and piglets following infection with a strain of epidemic influenza virus isolated in A.A. Smorodintsev's laboratory, from victims of the influenza epidemic of 1936 (Type A<sub>36</sub> strain, Leningrad).

## EXPERIMENTAL METHODS AND RESULTS

Experiments with white mice. We used 53 white mice of the same age and weight. Of these, 19 served as controls, 19 were inoculated with H. influenzae, and 15 with epidemic influenza virus.

The mice were inoculated under ether anesthesia, by intranasal instillation of infected material. The first few blood samples were taken from the tail vein. It was not, however, always possible to withdraw sufficient blood from the vein by this means, moreover, the vein often became empty after two or three punctures; for these reasons we then resorted to collecting blood flowing from the tail after the tip had been cut off. From three to seven hematological examinations were performed for each animal. In all, we performed 256 hematological examinations.

We determined the mean leucocyte count of blood withdrawn from the tail vein of mice of the control group, finding a value of 15,957, as compared with 13,967 in blood obtained by cutting off the tip of the tail; the mean leucocyte count for the control group was thus 14,962 per mm<sup>3</sup>.

We observed considerable variability in the leucocyte count of white mice. Other authors who have studied the hematology of mice found the same, and have recorded divergent mean values for the leucocyte count of white mice.

We inoculated 19 mice with a culture of H. influenzae. The dose of culture was sublethal, but gave rise

to pronounced symptoms of the infection. Only one of the group of 19 infected mice died.

The results of these experiments (mean values) are presented in Table 1.

TABLE 1

Mean Daily Leucocyte Counts of Mice Following Infection With *Hemophilus Influenzae* and Influenza Virus

Day after inoculation	Leucocyte count of mice		
	Infected with <i>H. influenzae</i>	Infected with 1/100 of a MLD of influenza virus	Infected with about 1 MLD of influenza virus
1st	10,897	20,607	16,561
2nd	13,702	11,844	12,962
3rd	13,915	12,775	10,149
4th	17,847	12,979	11,653
5th	15,254	12,700	12,155
6th	14,841	13,498	11,831
7th	14,330	12,135	14,419

It appears from the data of Table 1 that the leucocyte count of mice rises following inoculation with *H. influenzae*, reaching a maximum on the fourth day.

Fifteen white mice were inoculated with influenza virus; seven were given 1/100 of an MLD of virus, and eight received about 1 MLD. One mouse of the first group died. Of the eight mice given a dose of about 1 MLD, five died between the third and the seventh day after inoculation; autopsy revealed typical amicrobial consolidation of both lungs (no growth found on inoculating blood-agar with lung tissue). The mean values found for the leucocyte counts of both groups of infected mice show a definite tendency towards leucopenia (see Table 1).

Experiments with piglets. We used piglets weighing from 5 to 6 kg for our experiments. The leucocyte counts of individual uninfected animals varied widely (from 6400 to 9800; in one case 12,000, and in another 30,000), but the values for each individual animal showed much less variation. Inoculations were performed under ether anesthesia, by intranasal instillation (5 cc into each nostril) of a 10% homogenate of lung tissue of mice infected with Type A virus. Eight animals were treated in this way. We gave 1-2 instillations per diem (morning and evening). The total number of instillations amounted to 36 (from 1 to 7 instillations per animal), given at intervals of 12-24 hours. Blood samples were taken from ear veins. The body temperature of the animals was measured at the time of blood sampling. The first symptoms of disease were observed two to four days after inoculation; the animals became inactive and refused food; their respiratory rate rose, they coughed and appeared to have sore throats. Their temperature rose to 41-42° (normal temperature 38.5°).

The results of the hematological examinations are presented in Table 2.

In two cases the leucocyte count was unaffected (Table 2, Nos. 1 and 2) by inoculation with influenza virus; in two cases (Nos. 3 and 5) there was a transient and slight fall in leucocyte count; and in four cases there was a definite leucocytosis (Nos. 4, 6, 7 and 8).

We compared these hematological findings with those of anatomopathological, bacteriological, and virological examination of the animals, in order to find the reasons for these differences between the hematological responses of different animals. With this object, we killed the piglets at different times after infection. Thus, piglets Nos. 1 and 2 were killed on the first day after inoculation, Nos. 3 and 4 three days after inoculation, Nos. 5 and 6 four days after inoculation, and Nos. 7 and 8 were allowed to recover. We examined the lungs of the killed animals for pathological changes, and determined the virus content of the lung tissue; in addition, we made bacteriological cultures of the lung tissues and blood.

The lungs of all six animals showed regions of infiltration, the intensity of which increased with time after inoculation. In four cases out of six, cultures made by inoculation with the infiltrated lung foci proved to be

TABLE 2

Hematological Examination of Pigs Infected With Influenza Virus

Serial No. of animal	Leucocyte count (A and B)	Date of examination										Microflora of lungs	Blood cul- ture	Influenza virus con- tent of lungs
		9/7	9/8	9/9	9/10	9/11	9/12	9/15	9/17	9/20	9/21	10/1		
1	A { B {	9 600 38.7	9 800 39.6	—	—	—	—	—	—	—	—	—	Sterile	1:10 000
2	A { B {	7 600 39.5	7 700 40.1	—	—	—	—	—	—	—	—	—	“	1:10 000
3	A { B {	12 000 38.8	8 400 40.4	9 000 40.2	12 000 40.7	—	—	—	—	—	—	—	“	1:10 000
4	A { B {	9 200 39.0	8 000 39.6	11 200 39.8	12 800 39.9	—	—	—	—	—	—	—	Numerous and varied	1:10 000
5	A { B {	30 000 39.0	27 200 40.7	28 400 41.1	29 800 42.0	36 200 41.5	—	—	—	—	—	—	Sterile	1:10 000
6	A { B {	6 400 9 800	8 200 9 000	8 800 11 000	10 800 15 000	14 400 12 200	—	—	36 400	36 400	16 200	18 000	“	—
7	A { B {	—	39.4	40.7	40.5	41.5	40.0	39.5	40.5	39.2	39.0	39.8	—	—
8	A { B {	9 000 39.5	8 700 40.9	11 400 41.1	11 000 40.5	12 600 40.5	26 000 40.0	24 000 39.0	26 400 40.2	29 400 39.4	38 600 39.8	34 600 39.2	—	—

bacteriologically sterile, and in two cases (Nos. 4 and 6) a massive growth of mixed bacteria was obtained. Determination of the virus content of the lungs showed the presence of influenza virus in high titer in the infiltrated regions of the lungs of five out of six animals, and its absence in one case (No. 6).

Comparison of the results of hematological and bacteriological examinations of the lungs showed that of the four cases of amicrobial virus pneumonia the leucocyte count was unaffected in two cases and was slightly lowered in the other two (Nos. 1, 2, 3 and 5). A regular and considerable rise in leucocyte count was observed in all cases which were complicated by microbial infection (up to 14,400). The rise in leucocyte count was particularly large (up to 38,600) and prolonged (over 20 days) in piglets Nos. 7 and 8, which were allowed to recover. In these cases the leucocytosis was associated with severe pneumonia. These animals were sick for more than three weeks. They lagged behind other animals of the same age in body weight and growth rate. These animals were not autopsied.

Experiments with ferrets. Ferrets may be infected with influenza virus taken directly from a sick person. For this reason, we attached particular importance to the hematological examination of wild ferrets before and after their inoculation with the virus of epidemic influenza.

We made hematological examinations of 23 ferrets in all. We are not aware of any published data on the hematology of these animals, in sickness or health, and in this connection we encountered certain initial difficulties in the choice of the method for obtaining blood samples. Attempts at withdrawing blood from the tail veins after shaving off the hair were unsuccessful, and we were obliged to take blood from ear veins, which were nicked with a razor. This procedure permitted us to make five hematological examinations of each animal (one to two before infection, and two to three after infection). We made total and differential white cell counts.

An examination of 23 healthy ferrets before infection (40 analyses) proved that the animals have sufficiently stable amounts of leucocytes, most of them between 6,000 and 10,000 w.b.c per mm<sup>3</sup>; in one case, the count reached 16,600 and in another 18,200. The variations in the leucocyte count in individual animals were insignificant. A mean differential leucocyte count for healthy ferrets (according to our data) is as follows: leucocyte count 7866, band neutrophils 3% (236), segmented neutrophils 53% (4169), eosinophils 3% (236), basophils 0-1% (79), lymphocytes 27% (2124), monocytic cells 13% (1022). We use the term "monocytic cells," rather than "monocytes," because these cells possess a specific structure in the ferret, resembling the round macrophage cells encountered in the human.

Of the 23 ferrets, 13 were infected with influenza virus, eight of them receiving a lethal dose of virus (2 cc of 10% homogenate), two a small dose of virus, not lethal to a ferret (0.1 cc), and three were kept in contact with sick ferrets which had been given a lethal dose of virus.

The following hematological changes were seen in these 13 infected ferrets: leucocytosis in two cases, no change in four cases, and marked leucopenia in seven cases.

Anatomopathological, bacteriological, and virological examinations were made of all the infected ferrets which died or were killed. The results of these, and of the hematological examinations, are presented in Table 3.

It is evident from the data of Table 3 that leucocytosis was found in those ferrets suffering from pneumonia with heavy bacterial infection of the lungs (hemolytic streptococcus). No hematological reaction was found in the ferrets which had been in contact with sick ones. We could find neither bacteria nor influenza virus in the lungs of these animals. Evidently in such cases infection of the ferrets with influenza virus did not take place, for which reason there was no hematological reaction. Ferret No. 938 was only examined once, nine hours after inoculation. This animal was not subjected to hematological examination, and should not be taken into consideration.

The majority of the ferrets (seven animals) responded to infection with influenza with a definite leucopenia. Bacteria could not be found in their lungs or blood, and serological investigation of their lungs showed the presence of influenza virus in large amount. The results of hematological examination of some of these animals are presented in Table 4.

The following changes appeared in the differential white cell count of the ferrets after infection: fall in absolute neutrophil count in five cases out of seven; a shift to the left was observed for the nuclei, but it was

TABLE 3

Results of Anatomopathological, Bacteriological, Virological, and Hematological Examinations of Ferrets Infected With Influenza Virus

Serial No.	Infection date	Virus dose	Outcome	Pathological changes in lungs	Bacteriological exam of lungs	Blood culture	Virus content of lungs
1. Ferrets responding with leucocytosis							
955	10/29	2 cm <sup>3</sup>	Died 11/7	+++	Hemolytic streptococcus +++	Hemolytic streptococcus	1:100,000
923	9/27	2 cm <sup>3</sup>	" 10/2	+++	Proteus vulgaris +++		Not examined
2. Ferrets not giving any hematological response							
925	10/28	Placed in cage with a sick ferret	Killed 10/8	-	Sterile	Sterile	-
935	10/16	Ditto	" 10/20	-	"	"	-
924	9/29	"	" 10/8	±	"	"	-
938	10/15	2 cm <sup>3</sup>	" 10/17	++	"	"	1:10,000
3. Ferrets responding with leucopenia							
954	10/25	2 cm <sup>3</sup>	Died 11/1	+++	Sterile	Sterile	1:10,000
953	10/29	2 cm <sup>3</sup>	" 11/1	+++	"	"	1:10,000
937	10/16	2 cm <sup>3</sup>	Killed 10/19	++	"	"	1:10,000
922	9/27	2 cm <sup>3</sup>	" 10/2	+	"	"	1:10,000
958	10/29	2 cm <sup>3</sup>	Escaped 11/2	-	-	-	-
936	10/16	0.1 cm <sup>3</sup>	Killed 10/22	+++	Sterile	Sterile	-
934	10/16	0.1 cm <sup>3</sup>	Escaped 10/22	-	-	-	-

very small; juvenile and younger-than-juvenile neutrophils did not appear in the blood; eosinophils regularly fell or disappeared; in almost every case we observed a considerable fall in lymphocyte count during the first few days after infection (down to 104 per mm<sup>3</sup>, as compared with 2000 normally); the number of monocytic cells rose in most cases, sometimes by 40% (up to 2332 per mm<sup>3</sup>, as compared with 1000 normally).

The hematological reaction found in ferrets infected with influenza virus, uncomplicated by superposed bacterial infection, was basically analogous to that found by us in uncomplicated human epidemic influenza cases.

The hematological changes found by us in mice, piglets, and ferrets were basically of the same type. All these animals responded to pure virus infection with a leucopenia, and displayed leucocytosis only when there was superposed bacterial infection.

As has been stated above, the majority of piglets examined responded to influenza infection with a leucocytosis, whereas a definite leucopenia was found in the majority of ferrets examined. This difference may be ascribed to the much greater incidence of superposed secondary infection by various bacteria in the former case, due to the more severe injuries inflicted on their respiratory passages by the large amounts of inoculum introduced. The ferrets were given only one single inoculation, whereas the piglets were, in view of their low susceptibility to influenza virus, given repeated inoculations, up to seven in some cases. In general, each piglet

TABLE 4

Hematological Reaction of Seven Ferrets Which Gave a Leucopenic Response to Infection With Influenza Virus (the differential white cell count is given in absolute figures)

Examination date	Leucocyte count	Neutrophils		Eosino- phils	Basophils	Lympho- cytes	Monocytes
		band	segmented				
			Ferret No. 953				
10/26	7,200	144	3,960	72	72	2,160	792
10/27	6,700	134	3,819	67	-	1,943	737
10/29	6,800	204	3,808	-	-	2,108	680
10/29 Inoculation with a lethal dose of influenza virus (2 cm <sup>3</sup> )							
10/31	3,400	102	2,074	-	-	612	612
11/2	5,000	300	3,000	-	-	650	1,050
			Ferret No. 922				
9/27	7,000	-	2,450	560	-	2,660	1,330
11/27 Inoculation with a lethal dose of influenza virus (2 cm <sup>3</sup> )							
9/28	6,600	66	4,686	594	66	396	792
9/29	5,000	-	2,950	-	-	200	1,850
10/1	5,200	104	2,808	52	52	104	2,080
			Ferret No. 958				
10/26	6,800	612	3,468	-	-	1,700	1,020
10/27	6,900	138	4,692	69	-	1,173	828
10/29	6,800	-	4,080	136	-	1,360	1,224
10/29 Inoculation with a lethal dose of influenza virus (2 cm <sup>3</sup> )							
10/31	6,000	660	4,260	-	-	300	780
11/1	4,000	760	2,040	-	-	320	880
			Ferret No. 936				
10/14	16,600	-	12,284	-	-	2,158	2,158
10/15	15,200	304	10,488	-	-	2,584	1,824
10/16 Inoculation with a small dose of influenza virus (0.1 cm <sup>3</sup> )							
10/17	5,400	270	3,240	-	-	540	1,350
10/19	7,000	490	4,480	-	-	560	1,470
10/21	10,600	212	6,784	-	-	1,272	2,332

tolerated instillation of up to 70 cm<sup>3</sup> of infective homogenate into its respiratory passages, over the space of 2½ days.

#### SUMMARY

Data on hematological changes in 23 ferrets, 53 white mice, and eight piglets are presented. The changes took place before and after infection by the virus of Influenza Type A. Both the number of leucocytes and the leucocytic formulas were studied. Hematological data were compared with the data of pathologicoanatomical, bacteriological and virological investigation of these animals.

Observations showed that there was a tendency towards decrease of the number of leucocytes (in mice and pigs) or to considerable leucopenia (in ferrets) in virus nonmicrobic pneumonia. In presence of microbes regular pronounced and prolonged neutrophilic leucocytosis was noted.