a department of preventive medicine and public health and a department of bacteriology.

IT is reported in *The Scottish Geographical Magazine* that in a memorandum issued by the Council of the British Ecological Society, the establishment is recommended of a permanent R.A.F. unit for scientific work and of a Central Library of Air Photographs. Work which could be undertaken with the help of aerial survey includes the survey of inaccessible places, the periodic record of changing coastlines, the distribution of land liable to flooding, the establishment of correlations between environment and vegetation, primary surveys of vegetation, the distribution of certain animal species, the study of aerial plankton, and so forth. Many of the results would be economically valuable, and the aerial unit would necessarily facilitate the development of methods of survey and photography and the maintenance of highly trained personnel.

SPECIAL ARTICLES

THE TOXICITY OF INFLUENZA VIRUSES[1]

THIS investigation was initiated by the observation that intraperitoneal injection of certain allantoic fluids infected with various strains of influenza virus led to the death of some mice with characteristic lesions within 16 to 72 hours. Similar results were noted in a small series following intravenous administration of virus. On autopsy it was found that the blood vessels of extensive portions of the small intestines were engorged. Marked edema and distension of the gut was noted. The lumen of the duodenum, jejunum and ilium usually contained bloody mucous material which varied in color from pink to dark red. Occasionally small amounts of clotted blood were found in the stomach. The colon and rectum appeared normal, except in a few cases where petechial bleedings were observed throughout the length of the gastrointestinal tract.

The livers were usually of normal size with sharp edges. The surface showed a more or less distinct fine mottling, apparently caused by a pale yellow color of the periphery of the lobules while the centers retained their deep red color. The spleens were frequently enlarged and dark red. The lungs were generally normal in appearance at the early stages, but with increase of time between injection and death hyperemia and small areas of consolidation became visible in the case of some strains of the virus. Pleural exudate and ascites were not uncommon in animals which died 48 to 72 hours after injection.

Microscopically[2] the livers showed early widespread necrosis of the parenchyma in addition to marked hyperemia. Increased numbers of polymorphonuclear leucocytes were present. The spleens, likewise, were hyperemic and revealed distinct destruction of lmphocytes and necrosis of the Malpighian bodies in the earlier stages while after 48 hours the necrotic debris had been largely digested and regeneration had begun. The intestines were hyperemic and edematous. Other organs, aside from hyperemia, did not show any distinct changes.

Death from these acute lesions rarely occurred later than 72 hours after injection. Some surviving mice developed jaundice, particularly following injection of the F-12 strain of influenza A.[3] In these cases bilirubin was found in the urine, as determined by the Godfried modification of the Harrison spot test.[4] When such mice were sacrificed the subcutaneous fat tissue was found to be bright yellow. The livers were yellowish brown in contrast to the normal reddish brown, and on histological examination showed diffuse and focal proliferation of lymphoid and reticulo-endothelial elements. Focal necroses showing practically no peripheral reactions were common. The Malpighian bodies of the spleen were large and swollen, and marked perivascular reticuloendothelial proliferation was noted.

In the case of strains of influenza virus which produced a high incidence of the toxic signs, particularly the F-99 virus of influenza A,[5] death from lung lesions became apparent from the 5th day on. These lesions were identical with those following intranasal inoculation. Other, less toxic strains, caused mild pulmonary involvement or none at all, as has been observed previously.[6]

These results were ascribed to the action of influenza virus for the following reasons: Bacteriological examination of peritoneal fluid, liver and heart's blood proved negative. A search for pathogenic organisms in the intestinal contents yielded some cultures which on peritoneal injection killed mice but none of these animals died with the lesions described.

The toxic reactions were obtained in 2 strains of

[1] The work described in this paper was done under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Children's Hospital of Philadelphia.

[2] We are indebted to Dr. W. E. Ehrich for reviewing the microscopic sections.

[3] Isolated from the lungs of a fatal case of influenza. J. Stokes, Jr., and I. J. Wolman, *Internat. Clin.*, 1: 115–122, 1940.

[4] E. G. Godfried, *Biochem. Jour.*, 28: 2056–2060, 1934.

[5] Isolated from the lungs of an infant after a rapidly fatal respiratory infection.

[6] E. R. Rickard and T. Francis, Jr., *Jour. Exp. Med.*, 67: 953–972, 1938.

mice from different breeders. Injection of the following control materials did not produce these results: particulate components derived from emulsified normal allantoic sacs by high-speed centrifugation, normal allantoic fluids and allantoic fluids containing non-toxic influenza virus of an infectivity for chick embryos comparable to the toxic preparations. A few mice died following the injections of the normal particulate components because of the thromboplastic activity of such particles; blood clots could be extracted from the large vessels, whereas in the case of death from influenza virus preparations the blood was not clotted.

Most of the strains of influenza virus tested showed some toxicity which was demonstrable usually with undiluted or at most 4-fold diluted allantoic fluids only. Suspensions of infected mouse lung, like the particulate components of normal allantoic sacs, caused death of mice by their thromboplastic effect before the influenza lesions could develop. Allantoic fluids infected with the F-12 and F-99 strains of influenza A (both derived from fatal human cases[3,5]) and the S-15 strain of porcine influenza were outstanding in their toxicity. Less frequent toxic death was noted following the injection of the PR-8, WS and Melbourne strains of influenza A and the Lee strain of influenza B virus. The Weiss strain of influenza A, recommended for inclusion in the currently used influenza vaccines,[7] has not given such results as yet.

The agent could not be passed in emulsified liver by the intraperitoneal route on either a 2-day transfer starting with acutely damaged livers or by a 6- to 8-day-passage series beginning with livers from jaundiced mice. In both cases no signs developed in the 3 to 4 passages conducted. Propagation of the agent could not be ascertained in the peritoneal cavity or in the liver, confirming former reports by others.[6,8] The virus content decreased more or less rapidly following the intraperitoneal injection, as shown by titration in chick embryos of peritoneal washings or suspensions of livers. However, the lungs of mice escaping the early toxic death yielded rapidly increasing amounts of virus when the F-99 strain of influenza had been used for inoculation. This was consistent with the development of pulmonary lesions with resulting death in the later stages of the experimental period. In the case of the PR-8 and Lee strains, appreciable amounts of virus were present in the lungs soon after the intraperitoneal injection, but no macroscopic lesions developed and no increase of virus over the early titer was noted.

The toxic activity did not closely parallel any of the known properties of influenza virus. Preparations of various strains showing similarly high titers in the red cell agglutination reaction[9] or chick embryo infectivity tests varied in results from no toxic death to 100 per cent. fatality in 24 hours. This variation was noted also between different preparations of one strain of influenza virus. The use of more dilute inocula (0.2 ml of a 10^{-6} dilution) for the infection of chick embryos resulted frequently in allantoic fluids of greater toxicity than when more concentrated seed was employed (10^{-4} or more), although titration of virus and hemagglutinating capacity may have yielded similar titers. It seemed that the toxicity was a transitory property of the virus in that it appeared after the infectivity for chick embryos had reached its peak and faded again while the infectivity was still high.

The toxic principle behaved in every other respect like the influenza viruses. It was not dialyzable through Cellophane; it was removed from suspension by high-speed centrifugation at 20,000 r.p.m. in 20 minutes; it was adsorbed on and eluted from chicken red cells; it was neutralized by human influenza convalescent serum but not by serum taken before infection; mice immune to influenza A did not respond to injection of the homologous virus, while the administration of influenza B virus produced the toxic signs in similar numbers of animals, as in non-vaccinated controls, and conversely. Some of these properties of the F-99 strain are demonstrated in Table 1.

Thus far, only active virus has given the above re-

TABLE 1

RESULT OF INTRAPERITONEAL INJECTION OF VARIOUS PREPARATIONS OF THE F–99 STRAIN OF INFLUENZA A

Inoculum	Dilution of inoculum	Result of intraperitoneal injection (1.0 ml) Mouse No.							
		1	2	3	4	5	6	7	8
Original allantoic fluid	undiluted	T_1	T_1	T_1	T_1	T_2	T_2	T_2	3
	1:2	T_2	D_6	D_9	D_9	1	1	±	±
	1:4	D_6	D_6	3	2	±	±	±	±
20,000 r.p.m., supernate	undiluted	T_3	D_6	0	0	0	0	0	0
20,000 r.p.m., sediment	undiluted	T_1	T_1	T_1	T_1	T_2	0		
	1:2	T_2	D_7	D_7	2	±	0		
	1:4	T_3	D_6	D_6	D_6	D_6	D_9		
	1:8	D_6	2	±	±	±	±	0	0
Original allantoic fluid plus pre-infection serum		T_1	T_2	T_2	T_2	T_2	T_2	T_2	T_3
Original allantoic fluid plus F–99 convalescent serum		D^*_8	0	0	0	0	0	0	0

T_1, T_2 = toxic death within 24, 48 hours; D_6, D_7 etc. = death from pneumonia on 6th, 7th day; 3, 2, 1, ± = various degrees of pulmonary involvement found on autopsy 10 days after intraperitoneal injection; 0 = no lesions on the 10th day; * no macroscopical lesions found.

[7] Members of the Commission on Influenza, Board for the Investigation and Control of Influenza and other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, U. S. Army, *Jour. Am. Med. Asn.*, 124: 982–985, 1944.

[8] A. A. Smorodintseff and S. M. Ostrovskaya, *Jour. Bact. and Path.*, 44: 559–566, 1937.

[9] G. K. Hirst, *Jour. Exp. Med.*, 75: 49–64, 1942.

sults. Ultraviolet irradiation for short periods of time, heating to 56° C for 15 minutes, incubation at 37° C for 48 hours or addition of formalin 1:2000 destroyed the toxic effect. Sonic vibration for as long as 60 minutes left some of the toxic activity intact; so did repeated freezing and thawing at −70° C and 37° C, respectively. At 4° C the toxic agent was found to be stable for several weeks.

These results of intraperitoneal injections of influenza virus confirm earlier observations on the toxicity of influenza viruses.[10] They suggest that influenza virus may exert a toxic effect on various organs, although it is able to propagate only in the respiratory tract of the mouse. Similar results of a toxic effect have been noted recently by Evans and Rickard,[11] who produced lesions in the rabbit's cornea without demonstrable propagation of the influenza virus. It is felt that the toxicity demonstrated in these experiments may have a part in the pathogenesis of influenza in man. Since the strains of influenza virus mentioned have shown marked variation in their toxicity for mice it is conceivable that the difference in severity of influenza epidemics may depend on such factors. Although the etiologic agent of the 1918 pandemic has not been established and super-infections by various bacteria were widespread, it should be pointed out that severe hepatic damage was noted by some investigators in a large percentage of fatal cases studied during that time.[12] Toxic properties of certain rickettsial and viral agents have been described previously by Gildemeister and Haagen[13] and by Rake and Jones.[14]

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IMMUNOGENETIC CONSEQUENCES OF VASCULAR ANASTOMOSES BETWEEN BOVINE TWINS[1]

ALMOST thirty years have passed since Lillie[2] used the demonstrated union of the circulatory systems of twin bovine embryos of opposite sex to explain, on an endocrine basis, the frequent reproductive abnormalities of the female twin. Since the appearance of Lillie's paper, the freemartin, as the modified female is called, has become an important example of the effects of hormones on sex-differentiation and sexual development in mammals.[3] Consequences other than endocrinological of nature's experiment in parabiosis have, however, received little attention.

Estimates of the frequency of identical as compared with fraternal twinning indicate that the former is relatively rare in cattle.[4] Tests for inherited cellular antigens in the bloods of more than eighty pairs of bovine twins show, however, that in the majority of these pairs the twins have identical blood types. Identity of blood types between full sibs not twins is infrequent, as might be expected from the large number of different, genetically controlled antigens[5, 6] (now approximately 40) identified in the tests. If, therefore, the frequent identity of blood types in twin pairs can be explained neither as the result of monozygotic twinning nor as chance identity between fraternal twins, nor as the sum of these two factors, it is evident that some mechanism is operating to produce frequent phenotypic identity of blood types in genetically dissimilar twins. The vascular anastomosis between bovine twins, known to be a common occurrence,[2] provides an explanation.

Three additional, independent sources of evidence help to define the action of this mechanism. (1) One twin sire failed to transmit to any of his twenty progeny certain of the antigens found in his blood. In other words, the genotype of this bull as determined from his progeny appeared to lack factors responsible for some of the antigens found in his phenotype. Tests showed that cells containing these antigens could have been derived from his twin, whose genotype did contain the necessary factors. (2) In a case of superfecundation in cattle, involving twins of opposite sex and by different sires,[7] the twins had identical blood types, each possessing two antigens the genetic factors for which could not have come from his own sire or from the dam. Cells containing these

[10] G. Henle and W. Henle, SCIENCE, 100: 410–411, 1944.
[11] C. A. Evans and E. R. Rickard, *Proc. Soc. Exp. Biol. and Med.*, 58: 73–74, 1945.
[12] B. Lucke, T. Wight and E. Kime, *Arch. Int. Med.*, 24: 154–237, 1919.
[13] E. Gildemeister and E. Haagen, *Deutsch. med. Woch.*, 66: 878–880, 1940.
[14] G. Rake and H. P. Jones, *Jour. Exp. Med.*, 79: 463–486, 1944.

[1] From the Departments of Genetics (No. 346) and Veterinary Science, University of Wisconsin, in cooperation with the Bureau of Animal Industry, U. S. Department of Agriculture. This is part of a program aided by grants from the American Guernsey Cattle Club, the Holstein-Friesian Association of America, the Rockefeller Foundation and the Wisconsin Alumni Research Foundation. Appreciated contributions to various phases of the investigation have been made by Professor M. R. Irwin, C. J. Stormont and Mary W. Yeas. This study has been possible only through the generous cooperation of workers at many state experiment stations and of numerous private breeders of cattle.

[2] F. R. Lillie, SCIENCE, 43: 611–613, 1916.
[3] See "Sex and Internal Secretions," edited by Edgar Allen (Williams and Wilkins, Baltimore, 1939), for general discussions of and references to the literature on the freemartin.
[4] D. Sanders, *Zeit. für Züchtung B*, 32: 223–268, 1935.
[5] L. C. Ferguson, *Jour. Immunol.*, 40: 213–242, 1940.
[6] L. C. Ferguson, C. Stormont and M. R. Irwin, *Jour. Immunol.*, 44: 147–164, 1942.
[7] A description and discussion of this case will be published elsewhere by Mr. B. H. Roche, who called it to our attention and provided us with blood samples from the animals involved.