COMPARATIVE SEROLOGICAL STUDIES ON TALFAN AND TESCHEN DISEASES AND SIMILAR CONDITIONS

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A POLIO-ENCEPHALOMYELITIS affecting young pigs in Britain has lately been described by Harding, Done and Kershaw (1957) under the name of Talfan disease. The disease clearly showed similarities to Teschen disease, an infection of pigs well known in central Europe and Madagascar, and to poliomyelitis suum (Bendixen and Sjolte, 1955). It seemed highly probable that the Talfan virus, like Teschen virus (Larski, 1955; Mayr and Schwobel, 1956) would prove to be propagable in tissue culture: if so a ready means of serological comparison of these viruses would be at hand. Our expectations were quickly realized and the results of cross-neutralization tests are here briefly reported.

MATERIALS AND METHODS

The virus

Talfan.—Virus in the form of infected pig brain was available from animals inoculated in the transmission experiments described by Harding *et al.* (1957). The virus was propagated in tissue culture from 1st and 2nd pig passage material.

Teschen.—Dr. E. Traub (Tübingen, Germany) kindly sent Teschen virus (Konratice strain) at the 2nd and 58th subculture level. The latter was used in these experiments.

Sera

Talfan.—Sera from one hyperimmunized pig and several pigs convalescent after experimental infections were available.

Poliomyelitis suum.—Pig sera immune to poliomyelitis suum were kindly provided by Dr. Aa. Thordal-Christensen (Copenhagen).

Teschen.—Hyperimmune anti-Teschen pig serum was very kindly provided Dr. D. Horstmann (New Haven, U.S.A.) (1 Swedish and 3 Czechoslovak strains); other Teschen immune pig sera by Dr. E. Traub and Dr. F. Patoĉka (Prague) and poliovirus serum from immunized monkeys by Dr. J. O'H. Tobin.

Enzootic ataxia (Done 1957).—Serum was available from a naturally occurring case of the disease in an Essex pig. The serum was obtained 1½ years after the onset of clinical disease.

Tissue culture methods

Roller tube tissue cultures of pig kidney were used for this investigation. Fragments of kidneys from pigs of various ages were either explanted into a plasma clot on to the wall of a test tube (5 explants per tube) or were trypsinized by the method of Dulbecco modified by Balducci, Zaiman and Tyrrell (1956) and allowed to grow until a sheet of cells formed. The medium used for experiments with virus was composed of 5 per cent inactivated horse serum; 0·25 per cent lactalbumin hydrolysate; 40 per cent bovine amniotic fluid; 54·75 per cent Gey's solution; 100 units/ml. sodium penicillin G; 100 μg/ml. dihydrostreptomycin; (in cultures with plasma) 0·1 mg./ml. soya bean trypsin inhibitor and 0·01 mg./ml. phenol red.

Neutralization tests

The medium was removed from well-grown cultures of either kind and replaced by 0·8 ml. of fresh medium. Of the serum under test 0·1 ml. was then added to each of a group of 9 tubes and the cultures allowed to rotate in the drum for 10–20 min. Then dilutions of virus were added to groups of 3 tubes ; thus each serum was set up in triplicate against 3 dilutions of virus. The tubes were observed for 8 days. Previous experiments showed that if Talfan virus was present it would destroy the cultures within 1–8 days

EXPERIMENTAL

Virus derived from a case of Talfan disease was passaged ten times in pig kidney cultures. Fifth pass material was used as stock for subsequent experiments. The titre of virus at each pass tested in baby pig kidney was between 10^6 and $10^{7.5}$ tissue culture infectious doses ($T.C.I.D._{50}$) per ml. Adult pig kidney was less sensitive to virus. The virus destroyed the cells in 1–8 days, according to the dilution used as inoculum. Attempts to grow the virus in guinea-pig kidney, rat embryo, rabbit kidney cultures and in human liver cells (Chang line) and HeLa cells were unsuccessful, but the decay rate of virus in such cultures was very slow.

Talfan virus resisted heating at 56° for 3 hr. with a diminution of titre of less than 1000 times. The end-point of the titration was not reached, however, and the loss of virus may have been several logs less. Passes were made from cultures infected with heated virus, which showed that the destruction of the cells was due to live virus and not a toxic effect of killed virus. This virus also withstood 20 per cent ether treatment overnight at 4° with no significant loss of titre.

Talfan tissue culture virus at the 2nd and 5th subculture levels was inoculated intracerebrally into 3½-week-old litter-mate pigs as shown in Table I. The resultant disease differed from that produced experimentally with freshly isolated virus (Harding *et al.*, 1957) only in the higher morbidity rate and shorter incubation period ; clinically and histopathologically it was identical with Talfan disease.

Brain and cord emulsion from a pig killed at the onset of clinical disease (PD 3160) on the 6th day was inoculated into a further 4 piglets in which typical Talfan disease resulted. It was presumed that the very short incubation period

TABLE I.—*Experimental Infections in Pigs with Tissue Culture Talfan Virus*

Pig No.	Inoculum	Days to clinical disease	Days to death	Killed/Died	Histological result
PD 3184	5th pass tissue culture Talfan virus	10	14	K*	+
PD 3186		11	16	K*	+
PD 3187		15	16	K*	+
PD 3199		11	18	D	+
PD 3152	None	—	0	K	—
PD 3160	2nd pass culture virus	6	6	K	+
PD 3181		7	10	D	+
PD 3210	None (contact control)	—	21	K	—
PD 3209	Emulsion of C.N.S. of PD 3160	10	15	K*	+
PD 3213		10	15	D	+
PD 3214		10	13	K*	+
PD 3288		11	50	K	+
PD 3233	None (contact control)	—	28	K	—

* Killed *in extremis*.

seen with 2nd pass tissue culture virus was a function of the virus concentration in the inoculum rather than due to a fundamental change in the virus.

Relationship to Teschen Disease

The cytopathic effect of Talfan virus on pig kidney cultures is similar to that described by Mayr and Schwobel (1957) for Teschen virus. Talfan virus also resembles their virus in its high resistance to heat and to ether.

The disease produced by experimental infection of pigs with tissue culture Talfan virus more closely resembles classical Teschen disease in its high morbidity and mortality and shorter incubation period than Talfan disease as seen in Britain or poliomyelitis suum as described in Denmark. This parallels the observations by Mayr (1957) on Teschen virus.

Neutralization tests in tissue culture (Table II) showed that the cytopathic effect of Talfan virus was neutralized by 6 samples of Teschen antisera obtained from different countries, by poliomyelitis suum antiserum (Bendixen and Sjolte, 1955) from Denmark and by enzootic ataxia serum. It was not neutralized by human poliomyelitis antiserum of any of the 3 types. Talfan antiserum also neutralized Teschen virus in culture. Differences in potency between the various sera, obtained under varying conditions, are not thought likely to be of great significance.

TABLE II.—*Neutralization Tests with Talfan and Teschen Disease Viruses in Tissue Culture*

Virus	Serum	No. of LD_{50} neutralized (logs)
Talfan	Talfan	1·7
Teschen	,,	3
Talfan	Teschen	
	(a) Konratice (Traub)	3
	(b) Konratice (Horstmann)	1·2
	(c) Reporyje	1·2
	(d) Vetisuci	⩾2
	(e) Swedish	⩾2
	(f) Patoĉka*	2·3
Talfan	Enzootic ataxia	⩾2
Talfan	Polio suum	2·2
Talfan	Polio type I	<1
	II	<1
	III	<1

* Serum diluted 1/100. Other sera diluted 1/10.

DISCUSSION

Neutralization tests in culture and experimental infections with tissue culture virus demonstrate that Talfan disease and Teschen disease are caused by identical or closely similar viruses, which also are closely related to or identical with " poliomyelitis suum " from Denmark.

We feel it important that attention should be drawn to this similarity. The disease described by Harding *et al.* (1957) under the name Talfan disease is milder

than the disease described under the title of Teschen, but its virulence seems to be similarly enhanced by tissue culture. No epidemics have so far occurred in Britain, but according to Patoĉka (personal communication) only sporadic cases were at first observed in Czechoslovakia, the disease now being endemic with occasional epidemic outbursts.

SUMMARY

A virus causing paralysis in pigs in Britain (Talfan disease), was propagated in cultures of pig kidney : cultures reproduced the disease in pigs when inoculated intracerebrally.

Talfan virus was neutralized by antisera active against strains of Teschen disease from Czechoslovakia, Sweden and Germany ; also by sera from a case of enzootic ataxia in Britain and of " poliomyelitis suum " from Denmark. Antisera against all 3 types of human polio virus failed to neutralize it.

A strain of Tescben virus from Germany was neutralized by Talfan antisera.

The virus of Talfan disease thus appears to be closely related to or identical with that of Teschen disease.

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