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HUMAN RABIES. II — SEROLOGICAL STUDIES AND INTRA-VITAM VIRUS ISOLATION

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SUMMARY

Seven cases of human rabies have been studied through corneal imprints, daily performed, for intra-vitam virological diagnosis. No positive results were obtained. Six cases were also examined for virus antibody presence in the patients' saliva collected daily during the hospitalization period. Most of the patients showed three distinct periods in the course of their disease: one initial period in which just virus was detectable, a second period when neither virus nor antibody could be detected; a third period when just antibody was present. One of the patients exhibited a stage when virus and antibody were simultaneously detected. Another patient did not have the period in which virus and antibodies were absent. Rabies virus could be recovered from all the six patients' saliva, and from three patients the virus was isolated 13, 4 and 3 days before their death. Aqueous humor and cerebro-spinal fluid proved negative for virus. Antibodies by the Indirect Fluorescent Test (IFAT), were detected in one of the four samples of aqueous humor studied, in 1:20 dilution. One, out of the five examined samples of cerebrospinal fluid, presented a 1:10 antibody titer, by IFAT. The initial serum IFAT antibody titer varied from 1:10 to 1:40 in five patients, all of which showed an increase of antibody titers during the hospitalization period. The final sera antibody titers varied from: 1:80 to 1:320.

From all seven cases of human rabies, virus was isolated from Ammon's horn, cerebral cortex, cerebellum and except for one not performed, from the Gasserian ganglion. Two out of five submaxillary salivary glands examined were positive for virus. From the patients' lungs, heart, adrenals and kidneys no virus could be recovered.

INTRODUCTION

Many Authors believe that rabies is invariably fatal (STEELE [22], WAGNER [23], WHITE [24]), whereas others state that recovery from this infection does occur (BELL [1], BOLIN [2], HARRIS [6], KITSELMAN [9], MEDICAL WORLD NEWS [14], NILSON [15], PASTEUR et al.[16], RELOVA [19]), or could occur with an appropriate supportive treatment (LOPEZ et al.[13]). No evidence of the recovery however, can be offered, since the diagnosis of recovered human and animal rabies has so far been based on just epidemiological and clinical data.

There has recently been reported the complete recovery of one case of human rabies

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whose diagnosis was mainly based on epidemiological, clinical and serum-neutralization data. No virological diagnosis was obtained despite the attempts to isolate rabies virus from brain biopsy snips, cerebrospinal fluid and saliva collected two weeks after the illness onset. Fluorescein-tagged antibody stains of the brain biopsy snips, saliva smears and corneal imprints failed to emit fluorescence.

The presence of antibody has been suggested as responsible for many negative virological tests (EMMONS [3], KENT & FINEGOLD [8], LODMELL et al.[12]) as well as for the inhibition of fluorescent antibody staining (GOLDWASSER & KISSLING [4], GOLDWASSER et al. [5]).

The lack of available data regarding the presence and persistence of rabies virus in saliva, as well as the occurrence of antibody titers high enough for virus neutralization in the saliva and in the blood has been a handicap to the development of sensitive, specific and rapid techniques for the intra-vitam diagnosis of human rabies. Intra-vitam human cornea test, although successfully used for the diagnosis of rabies in mice and foxes (SCHNEIDER [21]), has failed in cases of human rabies (HATTWICK et al.[7], REIS et al.[18]).

Further data on the elimination of rabies virus and on the presence of antibody in the saliva, are the essential requirements for the performance of dependable virological tests and, then, the necessary support for the diagnosis of both natural and experimental recovery from rabies.

MATERIAL AND METHODS

Patients

Seven cases of clinically diagnosed human rabies have been studied. All epidemiological data regarding these cases, such as age, sex, origin, site of bite, animal involved, incubation period, vaccinal treatment after exposure, period of hospitalization and course of disease are shown in Table I. Only one patient (I.S.) received antirabies horse serum for 10 days, during the hospitalization period.

The seven patients were submitted to supportive treatment to sustain their biological functions until their neurological control was considered unrecoverable.

Saliva

During the hospitalization period, saliva from six patients was collected daily with a sterilized cotton wad (2 cm x 2 cm x 2 cm) and then examined for rabies virus by the fluorescent antibody technique (FAT) and suckling mouse inoculation. Rabies infection in inoculated mice was confirmed by FAT. Sediment of centrifuged saliva samples were used for slide smears and stained by FAT. The presence of rabies antibodies was detected by the indirect FA test (IFAT), using human globulin anti-serum labelled with fluorescein isothiocyanate.

The cotton wad soaked with saliva was placed in 2 ml distilled water containing 2% horse serum, penicillin (500 U/ml) and streptomycin (3 mg/ml). The pH was adjusted to 7.2 with potassium phosphate buffer M/15.

Cornea test

During the hospitalization period, corneal imprints were taken daily and prepared for FAT (REIS et al.[18] and SCHNEIDER [21]).

Serum

Blood from six patients was periodically collected for antibody titration by IFAT. The patient receiving anti-rabies serum was not examined for antibody presence.

Cerebrospinal fluid and aqueous humor

During the hospitalization period, cerebrospinal fluid from five patients and aqueous humor from four patients were irregularly collected. These samples were examined for the presence of virus, by FAT and suckling mice inoculation, and for antibody by IFAT.

Autopsy

All patients were autopsied, histopatological studies being conducted on frozen sec-

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tions (5 u) of Ammon's horn, fragments of the cerebral cortex, cerebellum and Gasserian' ganglion stained by Shorr's technique. Imprints of Ammon's horn, cerebral cortex and cerebellum were stained by FAT. Frozen sections of submaxillary glands, heart, lungs, kidneys and adrenals were also obtained for histopatological and virological studies by FAT and suckling mouse inoculation.

RESULTS

Saliva

In three out of six cases examined, the virus was isolated from saliva 13, 4, and 3 days before death. The incubation period, in infected suckling mice, ranged from 7 to 15 days. The mortality rate among suckling mice inoculated with the infective saliva, was 73.38% in the period of virus elimination (Tables II and III).

Except for one patient simultaneously displaying virus and antibody, other who did not survive to the stage when just antibodies are present in the saliva and for another without the period with simultaneous absence of virus and antibody all the individuals showed three distinct periods in the course of their disease: one initial period in which just virus was detectable; a second period when neither virus nor antibody could be detected; a third period when just antibody was present (Fig. 1).

Only three saliva smears, from three patients, provided positive or suggestive results regarding the presence of virus, by FAT.

Antibody titers in the saliva varied from 1:5 to 1:40.

Cornea test

All cornea tests performed provided negative results.

Serum

The antibody titers observed during the hospitalization are shown in Fig. 2. The five patients displayed initial titers ranging from 1:10 to 1:40 and final titers ranging from 1:80 to 1:320.

Aqueous humor

The four aqueous humor samples proved negative for virus, three of them also being negative for antibody. The one found positive for antibody presented a titer of 1:20.

Cerebrospinal fluid

The five samples of cerebrospinal fluid were negative for virus, the presence of antibody being detected in only one of them, in a 1:10 dilution.

Submaxillary salivary glands

Submaxillary salivary glands from two patients out of the five ones examined were found to be positive, by FAT and suckling mice inoculation techniques (Table IV).

Gasserian ganglion — Cerebral cortex — Ammon's horn — Cerebellum

Biopsy snips from all those areas provided positive results by FAT and suckling mice inoculation techniques (Table IV).

Heart — Lungs — Kidneys — Adrenals

Rabies virus could not be detected, by the afore mentioned techniques in any of those organs (Table IV).

DISCUSSION

Our experiments regarding the presence of virus and antibody in the saliva from six rabies patients showed that the patients underwent three distinct periods:

1st period, when only virus was detectable (five patients out of six);

2nd period, when no virus nor antibody could be detected (four patients out of six);

3rd period, when antibody was detected (five patients out of six).

TABLE I

History of seven cases of human rabies, Minas Gerais, Brazil, 1973

Patient	Sex	Age (years)	Origin	Bite site	Animal	Inc. per. (days)	Vaccinal treatment	Hosp. date	Hosp. per. (days)	Length of illness
I.S.	M	24	Teófilo Otoni	hand	cat	66	none	02/20/72	13	21
A.L.F.	M	14	Itaúna	leg	dog	425	14 doses	03/13/72	20	23
J.D.S.	F	8	Belo Horizonte	leg	dog	81	none	04/22/72	13	16
M.G.F.	M	42	Alpercata	hand	dog	90	none	09/20/72	14	29
R.P.G.	M	14	Belo Horizonte	face	dog	12	4 doses	09/30/72	11	14
G.R.	M	11	Belo Horizonte	leg	dog	90	none	11/28/72	11	17
W.L.C.	M	40	Belo Horizonte	nose	dog	62	none	12/20/72	15	18

Inc. per. = Incubation period
Hosp. per. = Hospitalization period

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TABLE II

Mortality rate of suckling mice inoculated with saliva from rabies patients; during the period of virus elimination

Patients	No. of positive	No. of inoculated mice percent positive
A.L.F.	21/ 37	56.76%
J.D.S.	64/ 66	96.96%
M.G.F.	11/ 29	37.93%
G.R.	22/ 26	84.61%
W.L.C.	46/ 66	74.36%
R.P.G.	29/ 39	69.69%
TOTAL	193/263	73.38%

One patient presented virus and antibody, simultaneously, during the first seven days of hospitalization, after which only antibody could be detected and another did not reach the stage when just antibodies are present. The other patients were seen to undergo a stage presenting only virus, followed by another showing just antibody (Fig. 1). These results are consistent with the data generally observed in the course of any infectious process from which a sick animal recovers. In the first period, the agent is predominant; in the second, the agent and the antibody are in equilibrium and, in a third period, the antibody is predominant. However, there are cases when the course of human rabies is so short that there is not enough time for the onset of the third period, or even the second period mentioned above.

The absence of virus in the saliva of two patients on the first day of hospitalization, may be accounted for by insufficiency of the specimen collected. Actually, it is difficult to get the necessary quantity of saliva in this period, its decrease being either a natural consequence of the disease or induced by the treatment administered to the patients.

Although not detectable in the patients' saliva, in the last stage of infection, the virus could be isolated from two out of five submaxillary glands examined. These findings suggest that the antibody had greatly reduced the presence of virus in the glands.

Reports regarding the virulence of the rabies virus isolated from saliva samples have so far been inconsistent (LEPINE & GAMET [11]). WILLIAMS [25] considered the hu-

TABLE III

Intra-vitam rabies virus isolation in suckling mice

Patients	Samples of saliva		Mice inc. period of 1st positive saliva sample (days)	Intra-vitam virus isolation
	Total	Positive		
A.L.F.	16	5 (31.25%)	7	Positive (*)
J.D.S.	12	9 (75.00%)	9	Positive
M.G.F.	14	7 (50.00%)	7	Positive
R.P.G.	10	5 (50.00%)	15	Negative
G.R.	10	3 (30.00%)	14	Negative
W.L.C.	13	10 (76.92%)	10	Negative (**)

(*) Positive = Virus isolation confirmed before patient died.
(**) The first positive sample of saliva was the 5th one collected during the hospitalization period.

man saliva rarely infections, while LEACH & JOHNSON [10] estimated one infectious case out of every three. On the other hand, PAWAN [17] obtained infection in all rabbits inoculated with human saliva, whereas SABIN & RUCHMAN [20] had negative results in a similar experiment.

The infectivity of the human saliva is proportional to the period in which it is collected. The virus can be more easily recovered in the first week after the onset of the clinical signs, its detection being much less likely after the appearance of antibody. As regards the intensity of salivation it was observed that, during the clinical phase, there is a variable period with very little or, even, no salivation at all. The dilution factor should then be taken into consideration when virus isolation is intended; therefore, the

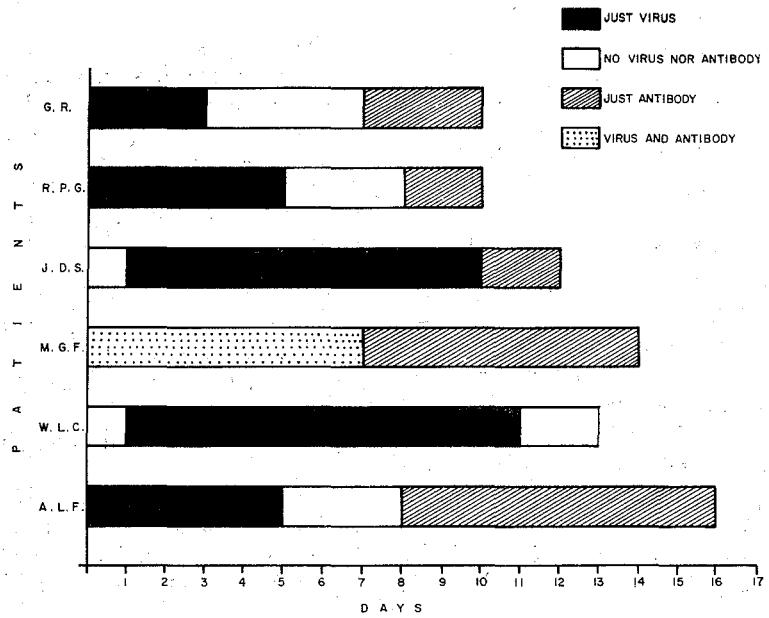

FIGURE 1 – Virus and antibody presence in saliva from six patients

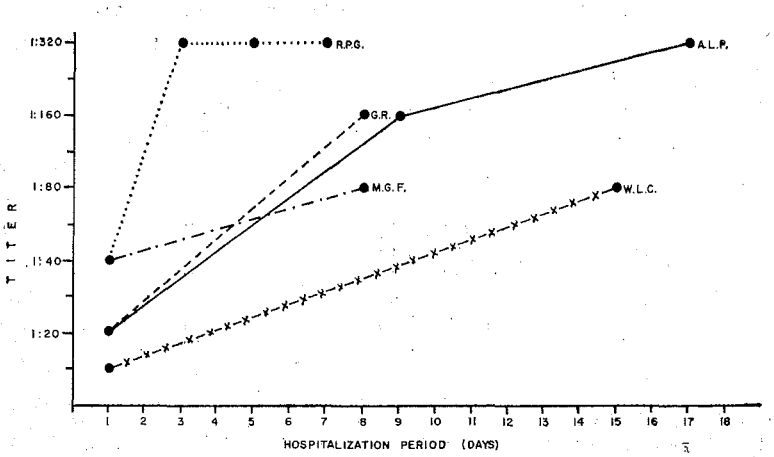

FIGURE 2 - Antibody titration, by IFAT, in the sera from five patients.

TABLE IV

Detection of rabies virus in the patient's organs

ORGANS / PATIENTS	CORTEX		AMMON'S HORN		CEREBELLUM		GASSERIAN GANGLION		SUBMAXILLARY SAL. GLAND		LUNG		HEART		KIDNEY		ADRENAL	
	FAT	Inoc.	FAT	Inoc.	FAT	Inoc.	FAT	Inoc.	FAT	Inoc.	FAT	Inoc.	FAT	Inoc.	FAT	Inoc.	FAT	Inoc.
I.S.	+	+	+	+	+	+	+	+	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
A.L.F.	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
J.D.S.	+	+	+	+	+	+	+	+	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
M.G.F.	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–
R.P.G.	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–
G.R.	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
W.L.C.	+	+	+	+	+	+	NP	NP	–	–	–	–	–	–	–	–	–	–

FAT = Fluorescent antibody test Inoc. = Suckling mouse inoculation NP = Not performed

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cotton wad must be weighed, before and after being soaked in saliva, so as to establish the suitable dilution level to be used in all cases.

A comparative study of the "Cornea Test", saliva smears (stained by FAT), and suckling mice intracerebral inoculation with saliva shows that the latter method is more reliable because of its higher sensitivity and specificity. Its great disadvantage lies in the fact that by this procedure it takes from 7 to 15 days to confirm the diagnosis. Nevertheless, it is our opinion that, for diagnosis confirmation, it should be included in every procedure concerning rabies virus recovery.

RESUMO

RAIVA HUMANA

II — *Estudos sorológicos e isolamento* "in vivo" *do vírus rábico*

Sete casos de raiva humana foram estudados pelo "Teste da Córnea", através de impressões de córnea coradas por imunofluorescência, feitas diariamente, durante o período de hospitalização. Todas as impressões de córnea foram negativas. Em seis casos foi pesquisada a presença de vírus e anticorpos em amostras de saliva, colhidas diariamente, durante o período de hospitalização. Exceto um paciente, que eliminou vírus e anticorpos simultaneamente, outro que morreu antes de atingir terceiro período e um terceiro que não apresentou o período em que vírus e anticorpos estão ausentes, todos os demais apresentaram três períodos diferentes no curso da doença: um período inicial em que apenas vírus foi encontrado; um segundo período em que nem vírus nem anticorpos foram evidenciados e um terceiro em que somente anticorpo estava presente. O vírus foi isolado da saliva em todos os seis pacientes. De três pacientes, o vírus foi isolado três, quatro e 13 dias antes da morte.

Amostras de humor aquoso (quatro) e de líquido cérebro-espinhal (cinco) colhidas irregularmente durante o curso da doença, apresentaram resultados negativos à pesquisa de vírus. Uma amostra de humor aquoso, entre as quatro estudadas, apresentou título de anticorpos, por imunofluorescência indireta, de 1:20. Uma amostra de líquido cérebro-espinhal, das cinco estudadas, apresentou título de anticorpos 1:10 por imunofluorescência indireta.

Cinco pacientes, dos seis examinados, apresentaram títulos de anticorpos iniciais no soro sangüíneo, que variou de 1:10 e 1:40, pela imunofluorescência indireta. Todos os cinco pacientes apresentaram aumento do título de anticorpos no soro, durante o período de hospitalização. Os títulos finais observados variaram de 1:80 a 1:320.

Duas das cinco glândulas salivares examinadas foram positivas para a presença de vírus. Pulmão, coração, rins e suprarrenal de cinco pacientes apresentaram resultados negativos para presença de vírus.

De todos os sete casos de raiva humana foi isolado vírus do Corno de Ammon, córtex cerebral e cerebelo. Também se isolou vírus dos seis gânglios de Gasser estudados.

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