FURTHER STUDIES ON KALA-AZAR

LEISHMANIA IN NASAL AND ORAL SECRETIONS OF PATIENTS AND THE BEARING OF THIS FINDING ON THE TRANSMISSION OF THE DISEASE

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Previous studies (1) of the nasal secretions of patients suffering from kala-azar yielded the following facts: (*a*) Smears from the nasal cavities of fifteen patients, when examined microscopically, revealed the presence of Leishman-Donovan bodies in nine cases. (*b*) Smears from the surface of the pharyngeal tonsil and from the saliva of one of these nine cases also showed the presence of leishmania. (*c*) The parenchyma of the tonsil of this latter case, at autopsy, was found to be massively infected with the parasites. (*d*) The nasal secretions of two patients were injected intraperitoneally into susceptible animals with the result that the animals became heavily infected, demonstrating that the parasites were viable.

These experiments showed for the first time that a rich source of infective material from a large proportion of patients with kala-azar was available for direct transmission of the disease. The present communication gives data confirming and extending the previous observations.

The medical literature contains almost no information concerning the presence or absence of leishmania in the secretions or tissues of the oral and upper respiratory tract of patients with kala-azar. The earliest study was that of Bentley (2) who in 1904 searched the sputum of patients, but failed to demonstrate the parasites. Carini (3) in 1911 and others subsequently found leishmania in the nasopharyngeal lesions of cases of espundia, a disease occurring in South America and caused by leishmania. This disease, however, has no similarity to kala-azar except that it is caused by a similar infectious agent. In 1913 Castellani (4) found leishmania in scrapings from chronic ulcers of the nasal mucous membrane of a case suspected of having kala-azar. Shortt (5) and his associates in 1932 examined various discharges and secretions of the body. They mentioned the saliva as a possible source of leishmania but did not examine it owing to the difficulty of using

cultural methods. The work of these latter investigators concerning leishmania in the urine and stools of patients was discussed in our previous paper (1).

Technical Details

The finding of Leishman-Donovan bodies in smears from the nasal secretions of patients with kala-azar is not difficult, but it demands careful preparation of the smears, a good microscope, an expert knowledge of the morphology of the parasite and persistence in searching. The parasites often are very few in number and may be scattered unevenly through the smear. Their small size and the presence in the nasal secretions of a variety of bacilli, cocci, cellular debris and albuminous material tend to render the parasites inconspicuous, and therefore prolong the time during which each oil immersion field must be examined before proceeding to the next.

We are convinced that the interested investigator can find the organisms in the smears without difficulty, but we are equally certain that search by the casual interne or the usual technician will be in vain. For the most part the smears on which these studies are based consisted of two or three preparations taken at one sitting only. A more vigorous search on several occasions might have yielded a higher percentage of positive findings.

There are several minor details in the preparation of the smears which are of value. An ordinary cotton-tipped culture swab was passed gently into the nasal cavity, and then onto the surface of a clean slide where the mucus and purulent material was spread thinly. These smears were allowed to dry in the air. Fixation with heat was avoided owing to its tendency to produce a finely divided precipitate of albuminous material. The slides were then flooded with Wright's stain and diluted with water in the manner ordinarily employed for blood smears. After washing, the smears were drained and allowed to dry in the air without blotting.

The animals used in these experiments were small Chinese field mice (*Cricetulus griseus*), commonly called hamsters, which were shown by Smyly and Young (6) to be highly susceptible to infection with *Leishmania donovani*. These animals, because of their cannibalistic tendencies, were kept in small, individual, wide meshed wire cages. In order to avoid cross-contamination among the animals and to render insect communication less likely, each cage containing either a control or an inoculated animal was suspended independently by a wire and separated from the others by an ample space of air. Under such conditions the hamsters survived and thrived for many months. Each animal was earmarked and in addition the cage was tagged with the animal number.

At times the intraperitoneal injections of saliva and nasal secretions were rendered difficult by the presence of thick mucus or crusts. Such material was prepared for injection by adding physiologic sodium chloride solution and then alternately filling and emptying a syringe onto which was attached at first large and then smaller needles. The feeding of infective material to the hamsters was accomplished by dropping the contents of a syringe into the open mouth and waiting for them to swallow while they were being held by an assistant.

Final examination of both control and inoculated animals was accomplished by autopsy. Smears were made of the spleen, liver and lymph nodes. These organs with other tissues were fixed in Zenker-formol (formalin 10 per cent) and after sectioning stained with hematoxylin and eosin.

The general plan was to inoculate four animals with each specimen of material. By such a procedure some allowance was made for accidental death of the animals and for individual differences in resistance and susceptibility to infection. Such a plan worked very well for the intraperitoneal injections of nasal secretions. However, the sputum or saliva of the patients when injected was much more likely to produce secondary pyogenic peritonitis which was fatal to a large proportion of the animals before sufficient time had elapsed for infection with leishmania to become manifest. As the result of these experiments it has been found that an interval of less than 45 days after intraperitoneal inoculation of either saliva or nasal secretions is insufficient for the infection with leishmania to be recognizable by the technique described. Hence animals which died earlier than the 45th day after inoculation were regarded as not surviving long enough for a satisfactory experiment. From our experience it would appear that the optimum time for the examination of animals after intraperitoneal inoculation with nasal secretions is about the 90th or 100th day, at which time heavy infection usually is present. Some animals show heavy infection as early as the 50th or 60th day.

EXPERIMENTAL

The nasal secretions of seven additional patients with kala-azar (Table I) have been examined microscopically. In three of these leishmania were present. This makes a total of twenty-two patients examined, in twelve of whom the parasites were found in the nasal secretions by direct examination. Smears from the tonsils of ten patients showed the parasites in three (Table V).

It will be noted that the nasal secretions of fourteen patients were inoculated intraperitoneally into hamsters (Table II). At the end of varying periods of from 45 to 200 days the animals were examined. From thirteen of the fourteen patients the nasal secretions were found to contain viable leishmania, evidenced by the infection of one or more of the hamsters of each group. The only patient in whom a negative result occurred was a small child from whom almost no nasal discharge could be obtained. Two animals only were inoculated from this patient and one of these did not survive long enough to be of value. There were three instances (Table V), Nos. P107, P115 and P103, in which direct examination of the nasal smears was negative but in which animal inoculation was positive.

Table III lists briefly the protocols of experiments concerning intraperitoneal inoculations into Chinese hamsters of saliva, sputum and of material from pharyngeal tonsils of patients suffering from kala-azar. It will be noted that sputum or saliva or both from thirteen patients was inoculated into animals. In five of these cases the animals died

TABLE I

*Data concerning Patients with Kala-Azar in Whom the Nasal Contents Were Examined for Presence of Leishmania**

Case No.	Age	Sex	Duration of symptoms	Edge of spleen below left costal margin†	State of the blood			Leishman-Donovan bodies in smears from		Globulin test on blood	Remarks
					R.B.C. per c.mm.	Hemoglobin per 100 cc.	W.B.C. per c.mm.	Spleen or liver	Nose		
	yrs.		*mos.*	*cm.*	*millions*	*gm.*					
P109	3	M	8	11.2	2.86	6.2	2,500	Many	Neg.	++++	Died. Pneumonia
P110	14	M	12	8.0	3.90	10.9	2,750	Few	Pos.	+++	Recovery
P111	5	F	7	13.8	2.54	7.5	1,700	Moderate number	Pos.	+++	Recovery
P112	18	F	9	24.0	2.87	8.2	1,200	Moderate number	Pos.	++++	Recovery
P115	11	F	17	23.0	1.43	4.1	1,700	Moderate number	Neg.	++++	Recovery
P116	13	F	19	17.0	3.52	7.7	2,200	Moderate number	Neg.	++++	Recovery
P103	22	M	1	3.0	3.89	10.0	2,400	Very few	Neg.	Neg.	Recovery

* Leishman-Donovan bodies were found in nasal smears from nine of fifteen previously reported cases.

† These measurements were from the mid-clavicular line at the left costal margin to the tip of the spleen.

early in the course of the experiment, too soon for infection with leishmania to become discernible. In the other eight cases two, Nos. P108 and P111, showed leishmania to be viable in the saliva or sputum. In two other cases, Nos. P78 and P112 (Tables III and V), material from the tonsils, obtained in the first case at autopsy and in the second by puncture of the tonsil during life, when inoculated demonstrated the infectivity of the parasites.

TABLE II

*Data concerning Intraperitoneal Inoculation into Chinese Hamsters of Emulsions in Normal Saline of Nasal Secretions from Patients with Kala-Azar**

Case No.	Hamster No.	No. of animals	Amount inoculated	Duration of experiment	History of animals	Presence or absence of leishmania in sections or smears of			No. of hamsters surviving less than 45 days†	
						Spleen	Liver	Lymph nodes	Control	Inoculated
			cc.	*days*						
P94	6, 8	2	1	79–93	Killed	Pos.	Pos.	Pos.	4	2
	9	1	1	93	Killed	Neg.	Neg.	Neg.		
	7, 10, 11	3	(Control) None	82–93	Killed	Neg.	Neg.	Neg.		
P78	14	1	1	45	Killed	Neg.	Neg.	Pos.	1	1
	15, 16, 17	3	1	62–118	Killed	Pos.	Pos.	Pos.		
	18, 19, 21	3	(Control) None	48–109	Killed	Neg.	Neg.	Neg.		
P87	29, 30	2	0.25	81–104	Killed	Neg.	Neg.	Neg.	None	None
	31	1	0.60	180	Killed	Pos.	Pos.	Neg.		
	37	1	0.80	193	Killed	Neg.	Neg.	Neg.		
	36	1	(Control) None	93	Found dead	Neg.	Neg.	Neg.		
P102	44	1	0.50	167	Killed	Pos.	Pos.	Neg.	None	3
	40	1	(Control) None	189	Killed	Neg.	Neg.	Neg.		
P104	58, 59, 71, 72	4	0.20–0.25	111–186	Killed	Pos.	Pos.	Pos.	2	2
	60	1	0.20	69	Killed	Pos.	Pos.	Neg.		
	55, 61	2	(Control) None	121–188	Killed	Neg.	Neg.	Neg.		
P105	78	1	0.20	106	Found dead	Pos.	Pos.	Neg.	None	None
	79, 80, 81	3	0.20	162–176	Killed	Pos.	Pos.	Pos.		
	97	1	(Control) None	118	Killed	Neg.	Neg.	Neg.		
P107	108	1	0.20	99	Found dead	Pos.	Neg.	Neg.	None	None
	109	1	0.20	151	Killed	Neg.	Neg.	Neg.		
	110, 111	2	0.20	200	Killed	Pos.	Pos.	Pos.		
	112, 114	2	(Control) None	48–87	Found dead	Neg.	Neg.	Neg.		

* Approximately equal quantities of nasal discharge and of normal salt solution were mixed together by means of drawing in and out of a syringe onto which was attached at first large and then smaller needles.

† 45 days was chosen arbitrarily as the minimum time after which a positive result could be expected in these experiments.

TABLE II—*Concluded*

Case No.	Hamster No.	No. of animals	Amount inoculated	Duration of experiment	History of animals	Presence or absence of leishmania in sections or smears of			No. of hamsters surviving less than 45 days†	
						Spleen	Liver	Lymph nodes	Control	Inoculated
			cc.	*days*						
P108	115, 117, 118	3	0.20–0.25	146–195	Killed	Pos.	Pos.	Pos.	None	None
	116	1	0.25	56	Found dead	Neg.	Neg.	Neg.		
	122	1	(Control) None	76	Found dead	Neg.	Neg.	Neg.		
P109	136	1	0.40	144	Killed	Neg.	Neg.	Neg.	None	1
	137	1	(Control) None	194	Killed	Neg.	Neg.	Neg.		
P110	123	1	0.25	146	Killed	Pos.	Pos.	Neg.	None	1
	124	1	0.25	195	Killed	Pos.	Neg.	Neg.		
	125, 126	2	0.20–0.25	195	Killed	Pos.	Pos.	Pos.		
	127	1	(Control) None	195	Killed	Neg.	Neg.	Neg.		
	144	1	(Control) None	113	Found dead	Neg.	Neg.	Neg.		
P111	145	1	0.25	143	Killed	Pos.	Pos.	Pos.	None	1
	147	1	0.30	112	Found dead	Pos.	Neg.	Pos.		
	152, 153	2	(Control) None	172	Killed	Neg.	Neg.	Neg.		
P112	169, 171	2	0.25	180	Killed	Pos.	Pos.	Pos.	None	None
	170	1	0.25	180	Killed	Neg.	Neg.	Neg.		
	172	1	0.25	101	Found dead	Neg.	Neg.	Neg.		
	162, 163	2	(Control) None	186	Killed	Neg.	Neg.	Neg.		
	164	1	(Control) None	123	Found dead	Neg.	Neg.	Neg.		
P115	197	1	0.30	173	Killed	Pos.	Pos.	Pos.	None	2
	199	1	0.30	113	Found dead	Pos.	Pos.	Neg.		
	205	1	(Control) None	173	Killed	Neg.	Neg.	Neg.		
P103	158, 159, 161	3	0.20	132–182	Killed	Neg.	Neg.	Neg.	None	None
	160	1	0.20	182	Killed	Pos.	Pos.	Pos.		
	155	1	(Control) None	182	Killed	Neg.	Neg.	Neg.		
	156, 157	2	(Control) None	150–169	Found dead	Neg.	Neg.	Neg.		

TABLE III

Data concerning Intraperitoneal Inoculations into Chinese Hamsters of Saliva, Sputum and of Material from Tonsils of Patients with Kala-Azar

Case No.	Hamster No.	No. of animals	Material inoculated	Amount inoculated	Duration of experiment	History of animal	Presence or absence of leishmania in sections or smears of		
							Spleen	Liver	Lymph nodes
				cc.	*days*				
	27, 28	2	Saliva	0.50	1	Found dead	—	—	—
P78	73	1	Scraping from cut surface of tonsil	0.20	59	Found dead	Pos.	Pos.	Pos.
	77	1		0.40	64	Killed	Pos.	Pos.	Pos.
	18, 19	2	None (control)	—	48–62	Killed	Neg.	Neg.	Neg.
P102	41	1	Saliva	0.25	19	Found dead	Neg.	Neg.	Neg.
	42	1		0.25	189	Killed	Neg.	Neg.	Neg.
P104	52, 53, 54	3	Saliva	0.25–0.35	155–209	Killed	Neg.	Neg.	Neg.
P105	100, 101, 103, 104	4	Saliva	0.20	17–137	Found dead	Neg.	Neg.	Neg.
P107	92, 93, 94	3	Saliva and sputum	0.30–0.40	1–6	Found dead	—	—	—
P108	119	1	Saliva	0.25	195	Killed	Pos.	Pos.	Pos.
	120	1		0.25	195	Killed	Pos.	Neg.	Neg.
	121	1		0.30	93	Found dead	Neg.	Neg.	Neg.
	122	1	None (control)	—	76	Found dead	Neg.	Neg.	Neg.
P109	133	1	Saliva	0.25	5	Found dead	—	—	—
	134	1		0.25	56	Found dead	Neg.	Neg.	Neg.
P110	138, 140	2	Saliva and sputum	0.25	194	Killed	Neg.	Neg.	Neg.
	141	1		0.50	76	Found dead	Neg.	Neg.	Neg.

TABLE III—*Concluded*

Case No.	Hamster No.	No. of animals	Material inoculated	Amount inoculated	Duration of experiment	History of animal	Presence or absence of leishmania in sections or smears of		
							Spleen	Liver	Lymph nodes
				cc.	*days*				
P111	148, 149, 150, 151	4	Saliva and sputum	0.30	137–172	Killed	Pos.	Pos.	Pos.
	152, 153	2	None (control)	—	172	Killed	Neg.	Neg.	Neg.
P112	165, 166, 167, 168	4	Saliva	0.25	1	Found dead	—	—	—
	193, 195	2	Abscess fluid from puncture of tonsil	0.20	1	Found dead	—	—	—
	194	1		0.10	175	Killed	Pos.	Pos.	Pos.
	162, 163	2	None (control)	—	186	Killed	Neg.	Neg.	Neg.
P115	200, 203	2	Saliva	0.40–0.50	7–9	Found dead	—	—	—
	201, 202	2		0.30	24–101	Found dead	Neg.	Neg.	Neg.
P116	208, 209	2	Saliva and sputum	0.40	1	Found dead	—	—	—
P103	47, 48	2	Saliva	0.25	10	Found dead	—	—	—

Having proved that resistant living leishmania were present in the nasal secretions of over 90 per cent of this small series of patients, it became of importance to know whether normal individuals could be infected easily when such secretions were administered by natural routes rather than by parenteral injections. Table IV gives brief protocols of a number of experiments in which nasal secretions, oral secretions and material from pharyngeal tonsils were introduced into the oral and nasal cavities of Chinese hamsters. This table also gives

data concerning inoculation of nasal secretions of patients with kala-azar into the nasal cavities of two normal human volunteers.

Perusal of the tables (Tables IV and V) reveals that the above experiments in many instances are still incomplete and in others that they have been only partially successful. Nasal secretions from the first case (No. P78) were inoculated into the nasal cavities of three animals. At the end of 59 days one of these animals (Hamster 22) was sacrificed. Search of the smears of the cervical lymph nodes and spleen revealed a few typical Leishman-Donovan bodies, but the infection was light and would have been missed had not a careful search been made. This experiment was of interest, but the slight degree of infection led to the prolongation of the time between inoculation and examination of the animals. The remaining two animals (Hamsters 23 and 24) together with two control hamsters (Nos. 25 and 26) were sacrificed after from 98 to 107 days. In none of these were leishmania demonstrable. However, three other animals (Hamsters 74, 75, 76) were fed with from 0.1 to 0.2 cc. of an emulsion in physiologic sodium chloride solution of material from the tonsil of the same patient. At the end of from 62 to 78 days all three were moderately heavily infected with leishmania.

Similar experiments with the nasal secretions and sputum of other patients, Nos. P87, P104, P105 and P107, are either incomplete or have been negative for transmission of the disease. Fifteen animals, Hamsters 128 to 131, 154, and 245 to 254, were the subjects of repeated feedings of nasal secretions from patients with kala-azar (Table IV). Four of these animals (Hamsters 129, 130, 154, 254), examined after from 97 to 107 days, were found to be uninfected with leishmania. One animal (Hamster 131) died too soon for the experiment to be of value. The remaining ten animals are still alive and will be examined at a later date.

Nasal secretions from two patients (Nos. P78 and P104) were inoculated (Tables IV and V) into the nasal cavities of two normal human volunteers (Nos. P67 and P72) on 3 successive days. These volunteers are well and show no signs of kala-azar 316 and 288 days respectively after the beginning of the experiment. Inasmuch as the incubation period of the disease in man is unknown, these volunteers will continue to be under observation for a considerable period of time.

TABLE IV

Data concerning Inoculation of Nasal and Oral Secretions and of Material from Tonsils of Patients with Kala-Azar into Oral and Nasal Cavities of Chinese Hamsters and Data concerning Inoculation of Nasal Secretions of Patients with Kala-Azar into Nasal Cavities of Two Human Volunteers

Case No.	Hamster or volunteer No.	No. of animals	Material inoculated	Amount inoculated	Route of inoculation	Duration of experiment	History of hamsters or of volunteers	Presence or absence of leishmania in sections or smears of		
								Spleen	Lymph	Lymph nodes
						days				
	22	1	Nasal secretion	Few drops	Nasal	59	Found dead	Pos.	Neg.	Pos.
	23, 24	2				98–107	Killed	Neg.	Neg.	Neg.
P78	74	1	Scraping from cut surface of tonsil	0.10 cc.	Oral	64	Killed	Pos.	Neg.	Neg.
	75	1		0.20 cc.		62	Found dead	Pos.	Pos.	Pos.
	76	1		0.20 cc.		78	Killed	Pos.	Pos.	Pos.
	P67 (volunteer)	1	Nasal secretion	Few drops	Nasal	Incomplete	Well	—	—	—
	25, 26	2	None (control)	—	—	107	Killed	Neg.	Neg.	Neg.
P87	32, 33	2	Nasal secretion	0.30–0.40 cc.	Oral	104–138	Killed	Neg.	Neg.	Neg.
	34	1		0.20 cc.		Incomplete	Living	—	—	—
	35	1	None (control)	—	—	Incomplete	Living	—	—	—
	36	1		—	—	93	Found dead	Neg.	Neg.	Neg.

P104	49, 50	2	Nasal secretion	5–6 drops	Oral	Incomplete	Living	—	—	—
	51	1		7 drops	Oral and nasal	119	Killed	Neg.	Neg.	Neg.
	63	1		0.20 cc.	Oral	Incomplete	Living	—	—	—
	64, 65, 66	3		0.10–0.20 cc.	Oral	101–170	Found dead	Neg.	Neg.	Neg.
	67	1		0.20 cc.	Oral and nasal	Incomplete	Living	—	—	—
	P72 (volunteer)	1		0.45 cc.	Nasal	Incomplete	Well	—	—	—
	55, 61	2	None (control)	—	—	121–188	Killed	Neg.	Neg.	Neg.
	69, 70	2		—	—	Incomplete	Living	—	—	—
P105	88, 90, 91	3	Nasal secretion	0.15–0.20 cc.	Oral	Incomplete	Living	—	—	—
	89*a*	1				173	Killed	Neg.	Neg.	Neg.
	97	1	None (control)	—	—	118	Killed	Neg.	Neg.	Neg.
	98	1		—	—	234	Found dead	Neg.	Neg.	Neg.
	105	1		—	—	Incomplete	Living	—	—	—
P107	95	1	Sputum	3 drops	Oral	Incomplete	Living	—	—	—
	96	1		4 drops	Oral and nasal	Incomplete	Living	—	—	—
	106, 107	2	Nasal secretion	Few drops	Oral	Incomplete	Living	—	—	—
	113	1	None (control)	—	—	Incomplete	Living	—	—	—

TABLE IV—*Concluded*

Case No.	Hamster or volunteer No.	No. of animals	Material inoculated	Amount inoculated	Route of inoculation	Duration of experiment	History of hamsters or of volunteers	Presence or absence of leishmania in sections or smears of		
								Spleen	Liver	Lymph nodes
						days				
P108; P110; P103; P111; P112; P115; P116*	128	1	Nasal secretion: P108 (2×); P110; P103 (2×); P112 (3×); P115; P116 Sputum: P111	Few drops at each repeated inoculation	Oral and nasal	Incomplete	Living	—	—	—
P108; P110; P103; P110; P111; P112; P115; P116	129, 130	2	Nasal secretion: P108 (2×); P110; P103 (2×); P112 (3×); P115; P116 Sputum: P110; P111	Few drops at each repeated inoculation	Oral and nasal	97–107	Killed	Neg.	Neg.	Neg.
P108; P110; P111	131	1	Nasal secretion: P108 (2×); P110 Sputum: P111	Few drops at each repeated inoculation	Oral and nasal	7	Found dead	—	—	—
P103; P112; P115; P116	154	1	Nasal secretion: P103; P112 (3×); P115; P116	Few drops at each repeated inoculation	Oral and nasal	98	Found dead	Neg.	Neg.	Neg.

P108; P110; P103; P111; P112; P110; P115; P116	132	1	None: Control for Hamsters 128, 129, 130, 131	—	—	164	Found dead	Neg.	Neg.	Neg.
P119; P120; P140; P141; P142; P143	245–253 254	9 1	Nasal secretions: P119; P120; P140; P141; P142; P143	Few drops at each repeated inoculation	Oral and nasal	Incomplete 103	Living Found dead	— Neg.	— Neg.	— —

* The studies tabulated in this and the subsequent horizontal columns were concerned with repeated inoculations from a series of patients with kala-azar.

TABLE V

Summary

Results of Microscopic Examination, of Intraperitoneal and Oral and Nasal Inoculation into Chinese Hamsters and of Nasal Inoculation into Human Volunteers of Material from Patients with Kala-Azar

Case No.	Microscopic examination			Results of intraperitoneal inoculation into hamsters of			Results of inoculation into nasal and oral cavities of hamsters of			Results of inoculation of nasal discharge into nasal cavities of human volunteers
	Material from liver or spleen puncture	Smears from nasal cavity	Smears from tonsils	Nasal discharge	Sputum or saliva	Material from tonsils	Nasal discharge	Sputum	Material from tonsils	
P94	Pos.	Pos.	—	Pos.	—	—	—	—	—	—
P95	Pos.	Neg.	—	—	—	—	—	—	—	—
P96	Pos.	Neg.	—	—	—	—	—	—	—	—
P97	Pos.	Pos.	—	—	—	—	—	—	—	—
P98	Pos.	Neg.	—	—	—	—	—	—	—	—
P78	Pos.	Pos.	Pos.	Pos.	Animals died too soon*	Pos.	Pos.	—	Pos.	Incomplete
P84	Pos.	Neg.	—	—	—	—	—	—	—	—
P99	Pos.	Pos.	—	—	—	—	—	—	—	—
P87	Pos.	Pos.	—	Pos.	—	—	Incomplete	—	—	—
P89	Pos.	Neg.	—	—	—	—	—	—	—	—
P102	Pos.	Pos.	Neg.	Pos.	Neg.	—	—	—	—	—
P104	Pos.	Pos.	Neg.	Pos.	Neg.	—	Incomplete	—	—	Incomplete
P105	Pos.	Pos.	Pos.	Pos.	Neg.	—	Incomplete	—	—	—
P107	Pos.	Neg.	—	Pos.	Animals died too soon*	—	Incomplete	Incomplete	—	—
P108	Pos.	Pos.	Neg.	Pos.	Pos.	—	—	—	—	—
P109	Pos.	Neg.	Neg.	Neg.	Neg.	—	—	—	—	—
P110	Pos.	Pos.	Neg.	Pos.	Neg.	—	—	—	—	—
P111	Pos.	Pos.	Neg.	Pos.	Pos.	—	—	—	—	—

P112	Pos.	Pos.	Pos.†	Pos.	Animals died too soon*	Pos.	—	—	—	—
P115	Pos.	Neg.	Neg.	Pos.	Neg.	—	—	—	—	—
P116	Pos.	Neg.	—	Animals died too soon*	Animals died too soon*	—	—	—	—	—
P103	Pos.	Neg.	—	Pos.	Animals died too soon*	—	—	—	—	—
Total..22	22 pos.	12 pos. 10 neg.	3 pos. 7 neg.	13 pos. 1 neg.	2 pos. 6 neg.	2 pos.	1 pos. 4 incomplete	1 incomplete	1 pos.	2 incomplete

* 45 days was chosen arbitrarily as the minimum time after which a positive result could be expected in these experiments.

† This positive smear was from material obtained by puncture of the tonsil.

DISCUSSION

The subject of the natural mode or modes of transmission of kala-azar has been one of greatest importance since the nature of the disease was first clearly recognized by Leishman (7) and by Donovan (8) in 1903. There exist large areas of heavily populated districts in China, India and in the countries bordering on the Mediterranean Sea where the disease is prevalent, the mortality high and facilities for treatment exceedingly few. One of the most hopeful avenues for escape from the ravages of the disease would be to discover its mode of transmission and thereby aid in the eradication of the conditions favoring its spread. To solve this problem has been the chief aim of various kala-azar commissions, and in addition to them many independent investigators have pursued the problem in its various aspects. Chief among the theories has been that the sand-fly actively transmits both kala-azar and oriental sore in much the same manner as trypanosomiasis, etiologically a somewhat closely related disease, is transmitted by the tzetze fly. Hundreds of papers and scores of investigators have dealt with this aspect of the problem, attempting by every conceivable means to incriminate the sand-fly. Strong presumptive evidence supports this theory but in spite of exhaustive attempts it has not yet been shown that the sand-fly is an important agent in the transmission of the disease. Out of many hundreds of susceptible animals, exposed each one to scores or in some instances hundreds of bites of infected sand-flies, in only three instances (9, 10) has kala-azar and in one instance (11) oriental sore been thought to have been transmitted. Eleven human volunteers subjected themselves to hundreds of bites of sand-flies known to be infected but there resulted no transmission of the disease. In fact the large volume of excellent work on the subject gives strong testimony that the sand-fly is not an important agent in the transmission of the disease. It seems conceivable that some insect other than the sand-fly or even the sand-fly itself may be shown eventually to be an important intermediate host. The chief evidence for and against the transmission of leishmaniasis by the bite of the sand-fly as accumulated from the voluminous literature on the subject, is listed in Table VI.

The second theory, that of transmission by direct or indirect contact

TABLE VI

Facts for and against Transmission of Leishmaniasis by Bite of Sand-fly

For	Against
1. Sand-flies readily become infected by feeding on oriental sores or on patients or animals with kala-azar	1. Rare to find infected sand-flies even in the houses of kala-azar patients
2. Occasional sand-flies caught in nature have been infected with leishmania	2. Sand-flies usually feed only once on mammalian hosts, but may with difficulty be forced to feed a second or third time. It is only after the second or subsequent feedings that the buccal cavity contains leishmania
3. Correlation exists between site on the body of bites of sand-flies and of occurrence of oriental sores	3. Cases of kala-azar have occurred in individuals who have never been in contact with sand-flies
4. Skin of infected individuals may be heavily infected with leishmania	4. Sand-flies exist in many parts of the world where kala-azar is not known to occur
5. Wherever oriental sore or kala-azar is endemic sand-flies have been found	5. Cultures of skin where infected sand-flies had been feeding were negative for leishmania
6. Leishmania ingested by sand-fly undergo development into flagellates and invade the buccal cavity	6. Out of many hundreds of susceptible animals exposed each to numerous bites of infected sand-flies in only three instances has kala-azar and in one instance has oriental sore been thought to have been transmitted
7. Infected sand-flies when fed through a membrane on sterile fluid produce infection of the fluid with leishmania	7. No instance of transmission of disease resulted when eleven human volunteers were subjected to hundreds of bites of infected sand-flies
8. Inoculation into skin of man and dog of emulsion of infected sand-flies produced dermal leishmaniasis	8. When infected and normal animals have been kept in the same cage, free from ectoparasites, infection of the healthy animals has resulted
9. Three hamsters subjected to numerous bites of infected sand-flies over prolonged periods of time acquired leishmaniasis after an interval of over 400 days	
10. One healthy dog in the same cage with sand-flies and an infected dog acquired the disease	

TABLE VII

Facts for and against Transmission of Leishmaniasis by Direct or Indirect Contact

For	Against
1. Viable parasites have been found in intestinal and gastric mucosa and in urine and stools of infected individuals	1. Leishmania ordinarily do not go through their complete life cycle (flagellation) at 37°C. or in the body of man
2. Kala-azar is notorious as a family and a house disease	2. Only one of thirty-two hamsters fed 151 times with feces of infected hamsters acquired the disease
3. During epidemic periods spread of disease required intimate contact	3. One monkey fed repeatedly on feces of a patient with kala-azar failed to become infected
4. Presence of infected and healthy animals in same cage results in infection of the normal animal	4. None of thirty-two hamsters fed repeatedly on deposit from fresh centrifuged urine of kala-azar cases, acquired the disease
5. Susceptible animals readily acquire disease if fed infected material (organs, cultures, infected sand-flies or ticks)	5. Many cases of endemic kala-azar arise in which there is no history of contacts with infected individuals. It is well known, however, that heavily infected individuals may remain symptom-free at least for many months
6. One case in man presumably infected by accidental sucking into the mouth of infected material	6. Kala-azar, at least in many districts, appears to be a rural disease, many more cases coming from the outlying districts of villages than from within the confines of the village itself
7. The form of leishmania found in bodies of infected patients can produce infections when administered orally to animals	7. No rich source of infective material from patients with kala-azar has been demonstrated
8. Flagellation is not a necessary phase for the reproduction of the parasite or for its infectivity	
9. Some cases of kala-azar cannot be explained satisfactorily on the basis of insect transmission	
10. House-flies and dog fleas readily ingest and may 5 minutes later deposit in their dejecta living leishmania	
11. Parasites remain viable and may multiply in milk for periods of days or months	

TABLE VII—*Concluded*

For	Against
12. Leishmania may survive or even may be cultivated in presence of bacterial infection with cocci and bacilli	
13. Leishmania survive after marked changes have occurred in their chemical or physical environment	

infection, has fewer adherents and has been tested much less rigorously. The major points in the evidence both for and against this mode of transmission are listed in Table VII. One of the chief arguments against this theory has been that no adequate source of infective material from patients with kala-azar has been demonstrated.

The results of the experiments here reported remove this objection in that it has been demonstrated that patients, whether examined early or late in the disease, almost without exception discharge in their oral and nasal secretions viable and infective parasites. In some instances, Nos. P107, P115 and P103 (Table V), direct examination of the nasal secretions was negative, but animal inoculation was positive. Such findings indicate that although the number of organisms injected must have been exceedingly few, yet their pathogenicity was such that massive infection occurred. Furthermore, these experiments show that the Leishman-Donovan body exists and retains unaltered its infectivity even in the presence of various and sundry types of bacteria which in number greatly exceed the leishmania. Such facts demonstrate a degree of resistance of the parasite which has been too little appreciated.

Although we have succeeded in transmitting the disease by means of introducing nasal secretions from patients into the nose and mouth of a susceptible animal and by means of transferring as little as 0.1 cc. of an emulsion of material from the pharyngeal tonsil into the mouth of another susceptible animal, yet the results have not been successful uniformly. These studies have by no means demonstrated that the usual mode of transmission of kala-azar is through the agency of direct contact, but they have added new facts to knowledge of the disease which lend additional weight to such an hypothesis.

SUMMARY

The summary of our experiments (Table V) including data previously reported is as follows:

1. Twenty-two patients were studied, in all of whom the diagnosis of kala-azar was confirmed by puncture of the spleen or liver, with recovery of Leishman-Donovan bodies.

2. Microscopic examination of the nasal secretions revealed typical parasites in twelve (54.5 per cent) of the twenty-two cases.

3. Microscopic examination of smears from the pharyngeal tonsils of ten patients showed parasites in three (30 per cent).

4. The results of intraperitoneal inoculation into hamsters of nasal discharge from fourteen patients proved that the parasites were present and had retained their infectivity in thirteen (92.8 per cent).

5. Intraperitoneal inoculation into animals of sputum or saliva killed many animals before sufficient time had elapsed for the presence or absence of infection with leishmania to be demonstrated. However, in eight cases animals survived and in two of these (25 per cent) transmission of the disease had occurred.

6. Material obtained from the pharyngeal tonsils of two patients, when inoculated intraperitoneally into hamsters resulted in infection with leishmania in both instances.

7. Single inoculations, into the oral and nasal cavities of hamsters, of nasal discharge from five patients have resulted in transmission of leishmaniasis in one instance only, but these experiments are incomplete.

8. Repeated inoculations by the oral and nasal routes of hamsters and of two human volunteers with nasal secretions from patients with kala-azar have not yet been concluded, but to the present date have resulted negatively.

9. Emulsions of material from the pharyngeal tonsil of one patient, when fed to three hamsters in amounts of from 0.1 to 0.2 cc. on one occasion only, resulted in generalized infection with leishmania in each of the animals.

10. Evidence as obtained from the medical literature both for and against the transmission of kala-azar by direct or indirect contact and by means of the bite of the sand-fly is presented.

CONCLUSIONS

1. Patients with kala-azar, whether the symptoms of the disease are of short or of long duration, almost without exception have present in their oral and nasal discharges viable, pathogenic *Leishmania donovani*.

2. Evidence is presented which strongly supports a theory of transmission of kala-azar by means of direct or indirect contact infection.

3. Unequivocal proof of the important natural mode or modes of transmission of kala-azar has not yet been presented. Much more work must be done before a final solution to the problem can be accepted.

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