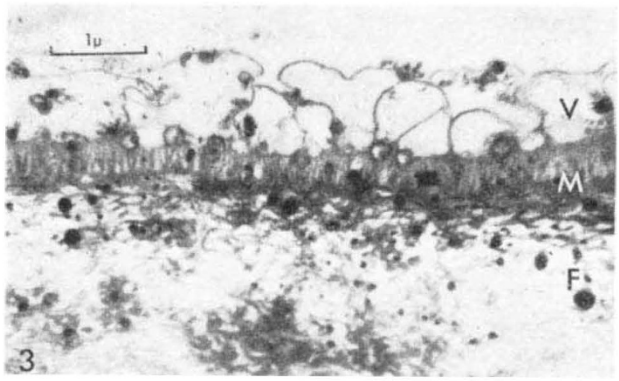

Fig. 3. Electron micrograph of vertical section of cuticle of cephalic lobe. F, Inner fibrous layer; M, middle dense layer; V, outer vesicular layer. Fixed glutaraldehyde, post-fixed osmic acid, stained lead and uranium.

surface layers of the mud[12–14] is by no means proof that pogonophores can exist on such food. Many other soft-bodied marine invertebrates have been shown to take up amino-acids and sugars from very dilute solutions, even though they also possess internal digestive systems[15–18]. We have not yet been able to show any uptake of glucose, and there is the question of very large molecules to consider. Nevertheless, it is tempting to suggest that although most animals may have some capacity for direct absorption of organic matter, the pogonophores have developed this capacity to the point where a gut is not needed.

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Epizootic of Simian Haemorrhagic Fever

Two epizootics of simian haemorrhagic fever (*SHF*) have been reported among quarantined Indian rhesus monkeys (*Macaca mulatta*). The first outbreak occurred in July 1964 at the Sukhumi Institute of Experimental Pathology and Therapy, USSR[1,2]; the second occurred in October 1964 at the US National Institutes of Health (NIH) quarantine colony[3,4]. The virus causing the NIH epizootic was isolated in cell culture and characterized[5]. Although the Soviet workers showed that the disease could be transmitted from monkey to monkey, they have not yet isolated the causative agent in either cell culture or small laboratory animals. This report describes the isolation in cell culture of the virus causing the Sukhumi epizootic and the serological relationship between the viruses causing the Sukhumi and NIH outbreaks.

After isolating the *SHF* virus strains causing the NIH outbreak we tested sera from monkeys involved in the Sukhumi epizootic by a complement fixation test with a 20× concentrated virus-infected cell culture antigen prepared from the prototype *LVR* 42-0/*M*6941 strain. Dr Robert J. Huebner kindly supplied us with thirty-six sera given to him by Professor V. D. Soloviev (Moscow) and Professor B. A. Lapin (Sukhumi). None of these sera reacted by complement fixation test. We were not convinced, however, of the lack of relationship because only two monkeys from the NIH epizootic reacted by complement fixation test. When these sera were checked by a more sensitive fluorescent-antibody test (unpublished observations of Tauraso, Aulisio and Shelokov) several reacted in an unfamiliar way with markedly subdued fluorescence. We did not feel that this represented non-specific reaction because the fluorescence occurred in that part of the cytoplasm and nucleus which we knew contained the viral antigen. It was possible that the reaction represented either a low concentration IgG antibody or a different class of antibody, possibly the earlier appearing IgM. We knew that low concentrations of complement fixation antibody occurred in some moribund animals when they were also viraemic[5].

Two of the Sukhumi sera which reacted by fluorescent antibody test were then inoculated intramuscularly into two rhesus monkeys housed in a specially constructed negative-pressure isolator (unpublished observations of Tauraso and Shelokov). One of the animals became ill 2 days after inoculation with a disease indistinguishable from that seen during the NIH epizootic. The second animal became ill 19 days after inoculation and it was not possible to determine whether its illness was caused by inoculation with a very small dose of virus or by exposure to the first sick monkey which was in the same isolator. The pathological findings in both monkeys were similar to those previously described for simian haemorrhagic fever occurring at NIH (ref. 4).

A viral agent which we have called the Sukhumi strain was obtained from each of these two monkeys when they were moribund by isolation in tube cultures of the *MA*-104 embryonic rhesus monkey kidney cell line using methods previously described[5]. After several passages in cell culture the virus caused typical clinical disease when inoculated into healthy rhesus monkeys and was isolated from the serum of these animals.

The Sukhumi strain was compared with three NIH strains, the prototype *LVR* 42-0/*M*6941, *LVR* 543/654, and *LVR* 42-0/*M*6941/*BS-C*-1. The Sukhumi, *LVR* 42-0/*M*6941 and *LVR* 543/654 strains produced similar cytopathic effects (CPE) in *MA*-104 cell cultures. The *LVR* 42-0/*M*6941/*BS-C*-1 strain having been passaged in *BS-C*-1 cell cultures produced a different CPE in *MA*-104 cells. This was characterized by a more rapid onset, granular appearance and lack of refractile cells characteristic of the CPE produced by the prototype *LVR* 42-0/*M*6941 strain[5].

By fluorescent antibody tagging, specific viral fluorescence was shown both in the nucleus and cytoplasm of cells infected with *SHF* virus. The four virus strains produced a similar picture. Their serological similarity was shown using the indirect fluorescent antibody technique with coverslips of *MA*-104 cells infected with each of the four strains, serum from a monkey surviving infection with the prototype strain (*LVR* 42-0/*M*6941) as the source of antibody, and fluorescein-conjugated anti-monkey globulin.

Table 1. COMPLEMENT-FIXATION TESTS WITH SERUM FROM MONKEY SURVIVING INFECTION WITH THE NIH STRAIN OF *SHF* VIRUS AND ANTIGENS PREPARED FROM THE SUKHUMI AND 3 NIH STRAINS

Antibody *SHF*	Antigen *SHF* virus strain No. 1*	No. 2	No. 3	Sukhumi
(42–0/*M*6941)	1024/64†	512/256	1024/64	2048/128

* Strain No. 1 = *LVR* 42–0/*M*6941 (prototype strain), No. 2 = *LVR* 543/654, and No. 3 = *LVR* 420/*M*6941/BS-C-1.
† Numerator, antibody titre; denominator, antigen titre (as determined by block titration)[5].

Table 2. PROPERTIES OF *SHF* VIRUS*

RNA
< 50 mμ
Chloroform sensitive
Acid (*p*H 3·0) labile
Heat (50° C) resistant
Increased heat inactivation in presence of divalent cations.

* Studies performed on prototype strain; *LVR* 42–0/*M*6941.

Table 1 shows the results of complement-fixation tests using immune monkey serum containing antibody to the prototype strain and the twenty times concentrated cell culture antigen previously described[5]. The Sukhumi strain could not be distinguished from the other strains in this "one-way-cross".

We conclude that the virus which caused the Sukhumi outbreak of *SHF* is similar to the virus strains that caused the NIH epizootic. The prototype virus strain (*LVR* 42-0/*M*6941) has been more completely characterized[5]. Table 2 summarizes the properties of the prototype *SHF* virus strain.

Although the Sukhumi serum samples travelled half way around the world we had no difficulty in isolating a virus from them. This stresses the potential danger in transporting specimens containing hazardous agents. The fact that two epizootics of *SHF* resulted from importation of rhesus monkeys from the same area in India to two distantly separated places in the world emphasizes again the known potential dangers involved in the transport and introduction of animals from one area to another. The more recent case in Germany[6–8] of fatal human disease resulting from contact with monkeys (*Cercopithecus aethiops*)* recently imported from Africa is a most dramatic and sad illustration of what would happen if the infectious agent is also communicable to man.

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Influence of Temperature on the Isometric Myograms of Cross Innervated Mammalian Fast Twitch and Slow Twitch Skeletal Muscles

THE operation of cross innervation of mammalian skeletal muscles has been described before[1,2] together with the observation that as a result of this procedure the normally fast twitch skeletal muscles contract more slowly and the usually slow twitch muscles contract more rapidly. Although, as a result of cross innervation, fast twitch muscles slow to an extent which renders their isometric twitch contractions indistinguishable from those of normal or self reinnervated slow twitch muscles, the contraction characteristics of cat cross innervated slow muscles have never been observed to become as rapid as in normal or self reinnervated fast twitch muscles. In addition, while the values of the tetanus twitch ratios of cross innervated fast muscles fall within the range found in normal mammalian muscles, the tetanus twitch ratio values of cross innervated slow twitch muscles are almost invariably high[1,2]. The cats on which these previous observations were made had been left for periods averaging approximately 1 yr between the aseptic operation and the final observations. It seemed possible that after a longer interval there might be a more complete transition of the slow twitch muscle. We have therefore studied three cats on which the cross innervation operations had been performed between 4 and 5 yr earlier. In two cats cross innervation of soleus (a slow twitch muscle) and flexor hallucis longus (FHL, a fast twitch muscle) had been performed, while in the third cat flexor digotorum longus (FDL) was used instead of FHL. The transition of all three fast muscles was complete; their times to peak tension had lengthened approximately three-fold to values found for normal soleus muscles. Their tetanus twitch ratios were also normal, averaging 4·6 (range in normal muscles, 4·0–6·9, mean 5·4). Even after 4 yr, however, the cross innervated slow twitch muscles were unambiguously different from normal fast muscles (Fig. 1). The times to peak tension had only fallen to approximately half the values found in the control muscles, and the tetanus twitch ratios were still high, averaging 7·0 (range in normal muscles, 3·6–5·7, mean 4·5). It therefore seems likely that in the cat the cross innervation of normally slow twitch mammalian muscle cannot produce a muscle with characteristics similar to normal fast muscle. It is, however, interesting that the characteristics produced are very similar to those observed in the soleus muscles of kittens within a week of birth[3,4].

It has been shown that the isometric twitches of normal mammalian fast and slow twitch muscle behave differently if their temperature is lowered from 38° C to 28° C (ref. 5). While the times to peak tension of both types of muscle are lengthened the maximum twitch tension of fast twitch muscles increases by approximately 25 per

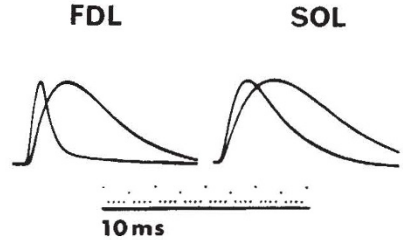

Fig. 1. Superimposed maximum isometric twitch contractions of self reinnervated and cross innervated FDL muscles (left hand side) and soleus muscles (right hand side) 4 yr after operation. The faster of the two FDL contractions is the self reinnervation as is the slower of the two soleus contractions. The amplifier gain has been adjusted to make the sizes of the twitch responses similar. Note the obvious difference between the self reinnervated FDL and the cross innervated soleus muscle, as compared with the more similar cross innervated FDL and self innervated soleus.