direkte Absorption von $^{14}CO_2$ im Messgläschen für die Szintillationszählung. (2) Die Messung des insgesamt absorbierten $^{14}CO_2$, was die Erfassung sehr geringer Mengen ermöglicht. Im Vergleich zu dem kürzlich von AMENTA und DOMINGUEZ[11] angegebenen Typ, ist das beschriebene Absorptionsgefäss in Konstruktion und Handhabung bedeutend einfacher[12].

Summary. A novel and simple kind of $^{14}CO_2$ trap is described. It can be used directly for subsequent scintillation counting. Solutions for optimal absorption and counting efficiency are indicated.

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Dengue-Like Viruses Isolated by an Improved Method

Antibodies for dengue viruses were demonstrated in the sera of patients with dengue-like fever in Jamaica during an outbreak in 1963 by GRIFFITHS and GRANT[1]. After many unsuccessful attempts to isolate the etiological agent in suckling mice, the following method was used with success.

The infectious material examined was the acute sera of patients with dengue-like fever which showed a significant rise of dengue antibodies in their convalescent sera. For the isolation, Roux bottles containing 8–10 million Bristol HeLa cells in monolayers were used. After inoculation of the serum, the cells were maintained in Eagle's medium with the addition of 2% calf serum. The maintenance medium was replaced with fresh medium at 5-day intervals. The medium collected was stored at – 20°C. The bulk of the cell culture medium collected from the same Roux bottle of inoculated cells was twice extracted with 20 Vol of acetone and dried with a vacuum pump. After the evaporation, the cell culture medium was reconstituted to the original volume with borate buffered saline, pH 9. The reconstituted cell culture medium was concentrated up to 150 times by the use of ultrafilter LKB 6300 A as described previously[2]. In the final preparation, the hemagglutinins were demonstrated with goose red blood cells according to techniques described by CLARKE and CASALS[3].

In the cell culture fluid of Bristol HeLa cells inoculated with the sera of two patients with dengue-like fever in 1963, hemagglutinins could be demonstrated by the use of the method described above. The optimal titres of hemagglutinins demonstrable in the concentrated and acetone-extracted cell culture fluids, were obtained at pH 6.6. HeLa cell culture fluids containing hemagglutinins were infectious for fresh Bristol HeLa cells in monolayers. Table I shows the titres of hemagglutinins in cell culture fluids for three passages of the two virus strains isolated.

Table I. Titres of hemagglutinins in the HeLa cell culture medium inoculated with the sera FV 1088 and FV 1119

Passage level	Day after inoculation	Titres of hemagglutinins at pH 6.6 FV 1088	FV 1119
1	5	< 10[a]	< 10
	15	160	40
2	5	< 10	< 10
	25	40	40
3	5	10	< 10
	15	40	40

[a] Figures denote the mean titres of four titrations in reciprocal values.

Table II. The results of the cross complement fixation test for the virus strains FV 1088 and FV 1119

Antigens	Serum Dengue 1	Dengue 2	Dengue 3	Dengue 4
Dengue 2	< 2/< 2	32/256	< 2/< 2	< 2/< 2
FV 1088	< 2/< 2	4/4	8/32	8/8
FV 1119	4/8	< 2/< 2	16/64	8/32

By the use of cross complement fixation it could be shown that the two viruses isolated in Bristol HeLa cell cultures belong to the group of dengue viruses. Virus strains FV 1088 and FV 1119 are closely related to dengue type 3 and dengue type 4 viruses, as can be seen from Table II. The final preparations of hemagglutinins were used in the complement fixation test as antigens.

BUCKLEY demonstrated on many occasions[4,5] that HeLa cells can be used successfully for the growth of dengue virus types 1–4. We believe that the method described in the present report could be used as a valuable expedient for the isolation of dengue viruses. The comparative ease with which hemagglutinins are prepared by our method offers new possibilities for the identification and the isolation of dengue viruses.

Zusammenfassung. Aus Seren von Dengue-Fieberkranken (Epidemie 1963) wurden zwei «Dengue-like» Viren isoliert. Die Isolierung der Stämme gelang in Bristol-HeLa-Zellen, die mit dem LKB 6300 A Ultrafilter dargestellt wurden. Die Identifizierung der Viren erfolgte mit der Reaktion der Komplementbindung.

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