totoxic substances either respond with greater mobilization of lipids to the liver following injection of LM, or that hepatotoxic agents interfere with utilization of mobilized fat brought to the liver. Our rats responded to a 24 hour fast by depletion of liver glycogen similar to that reported for the Wistar strain (13). The fact that hyperlipemia appeared in all the species tested indicates that the action of LM is not species or strain specific. The potency of crystalline LM, its activity in hypophysectomized rats and the failure of hypophysectomized rats to produce LM suggest the hormonal nature of this material. The increased purity resulting from crystallization was due largely to the removal of inorganic solids from the dialyzate. Preliminary studies indicate that the active material is a peptide that has neither vasopressor nor oxytoxic actions.

*Summary and conclusions.* (1) The active hyperlipemia inducing constituent of the dialyzate obtained from the plasma of horses administered cortisone has been crystallized and tentatively identified as a peptide. (2) Injection of 0.02 $\mu$g of crystalline material/kg IV induced marked hyperlipemia in intact mice, rats, guinea pigs, rabbits, dogs and humans as well as in hypophysectomized or adrenalectomized rats. (3) The animals used in this study were exposed to potential hepatotoxic agents.

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## Infectious Bovine Rhinotracheitis (IBR). I. Propagation of Virus in Cancer Cells of Human Origin (HeLa). (23255)

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Infectious bovine rhinotracheitis (IBR) virus was successfully grown in tissue cultures of bovine embryo kidney, testicle and lung by Madin and others(1), and carried by them through 37 serial transfers in kidney tissue. These authors, however obtained no indication of cytopathogenic action of the virus on HeLa, KB or L cells or chicken fibroblasts. The present communication reports propagation of IBR virus in HeLa cell cultures through 26 serial passages.

*Materials and methods. Virus strain*: The virus used in this study was obtained from Drs. J. A. Baker and J. H. Gillespie, Veterinary Virus Research Inst., Cornell University, Ithaca, N. Y., to whom we are grateful. It was received in this laboratory as a frozen lung specimen from an experimentally infected calf, sacrificed while showing symptoms of IBR. *Tissue cultures*: Trypsinized stationary tube cultures of bovine embryo kidney, lung and skin-muscle were prepared

by the method of Dulbecco and Vogt(2) in Earle's basal medium(3) containing 20% inactivated cow serum and lactalbumin hydrolysate(4). Renewal fluid consisted of solution 199(5). Stationary tubes of HeLa cells were cultivated in Eagle's basal medium (6) containing 20% inactivated horse serum. In early passages, HeLa cultures were renewed with Eagle's basal medium containing 5% chicken serum. Later, Ginsberg's medium(7) was substituted throughout, save for a few serial passages which were renewed with Eagle's containing 5% cow serum. Cultures were consistently renewed with 1 ml fluid and were uniformly infected with 0.2 ml bovine or HeLa cell tissue culture fluids. Tubes were observed daily until the cytopathogenic effect was complete in infected tubes but for not more than 7 days. Harvested cells and fluids were ground in a Tenbroek grinder and either subcultured on the same day or stored in the dry ice chest for future use. Titrations were made in either bovine embryo kidney or HeLa cell cultures, using 0.2 ml of each 10-fold serial dilution in 2 or 3 tissue culture tubes. The TC-$ID_{50}$ titers were calculated by the Reed and Muench formula(8). *IBR hyperimmune guinea-pig serum*: Five guinea pigs were hyperimmunized with bovine kidney grown virus as follows: two 1 ml intramuscular injections of an equal mixture of 3rd passage undiluted fluid and adjuvant mixture (Bayol F: 9 parts Arlacel A: 1 part) given one week apart followed, 3 weeks after the second injection, by a 2.0 ml interperitoneal injection of 7th passage undiluted fluid. The animals were bled 10 days later, their serum pooled and stored in the freezer. This serum-pool had a neutralizing titer of 1:32 against 100 TC-$ID_{50}$ of virus when tested in bovine kidney cultures. *Quantitative serum-neutralization test*: For virus identification, duplicate series of 10-fold dilutions of the virus preparation, beginning 1:5, were made in phosphate buffer solution. To the dilutions of one series, equal volumes of a 1:2 normal guinea-pig serum were added and, to those of the other, equal volumes of a 1:2 hyperimmune guinea-pig serum. Following incubation in the water-bath at 37°C for 1 hour, each dilution was inoculated into 2 tissue culture tubes in 0.2 ml volumes. For determination of serum neutralizing antibodies, dilutions of a standard virus preparation were mixed with equal volumes of undiluted normal calf serum and the serum under test, incubated and inoculated as above. Neutralization indices were derived from the difference between the log TC-$ID_{50}$ titers of normal and immune serum mixtures. *Experimental calves* were heifers of the Hereford breed, weighing from 700 to 900 lb, purchased from a region where clinical IBR was not known to have occurred.

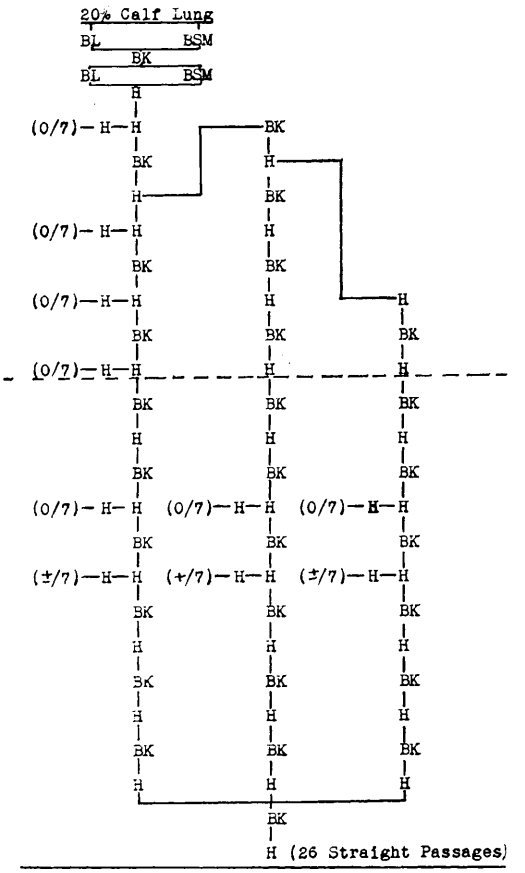


CHART 1. Passage History of IBR Virus in Bovine Tissues and HeLa Cell Cultures.

BL-BSM-BK = Bovine lung, skin-muscle or kidney cultures. H = HeLa cultures. (0/7)-(±/7)-(+/7) = Cytopathogenic effect absent, questionable or definite in 7 days. HeLa cultures above dotted line renewed with Eagle's basal medium containing 5% chicken serum; below dotted line, with Ginsberg's fluid.

*Results. Passage history of IBR virus in bovine kidney and HeLa cell cultures*: For

TABLE I. Representative Titers of IBR Virus at Various HeLa Passage Levels in Bovine Kidney and HeLa Cells.

| | HeLa passage level: TC-$ID_{50}$ titers | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Titration in: | 6 | 7 | 8 | 9 | 10 | 15 | 23 | 25 |
| HeLa | $10^{-4.5}$ | $10^{-3.5}$ | N.T.* | $10^{-4.5}$ | N.T. | N.T. | $10^{-2.5}$ | $10^{-4.5}$ |
| Bovine kidney | $10^{-5.5}$ | $10^{-6.0}$ | $10^{-5.5}$ | $10^{-6.0}$ | $10^{-5.0}$ | $10^{-5.0}$ | $>10^{-4.5}$ | $10^{-5.0}$ |

* Not tested.

the sake of clarity, this is given diagrammatically in Chart 1. The original material, 20% calf lung suspension in phosphate buffer solution, was inoculated simultaneously into bovine embryo lung and skin-muscle cultures. A cytopathogenic effect became evident within 24 hours in both types of cultures and most cells had sloughed off the glass by the 3rd day. A second passage in bovine kidney cells was ready for harvest in 48 hours, whereas a third, in bovine lung and skin-muscle cultures, was again ready and harvested in 3 days. A pool of this latter passage was used to initiate the adaptation of the virus to HeLa cells. Upon the very first HeLa passage, the cells rounded and clumped up within 24 hours and, by the 3rd day, had all fallen off the glass. In the 2nd HeLa passage, however, very little change was observed in 4 days although virus was readily recovered by subinoculation into bovine kidney. A 3rd straight transfer showed no cellular alteration whatever in 7 days and was discarded. From the bovine kidney subculture fluid, essentially alternating passages between HeLa and bovine kidney cells were made. As shown in Chart 1, one main and 2 side lines of alternating transfers were carried down beyond the point where second HeLa passages showed definite cytopathologic changes. A HeLa pool of the 3 passage lines was finally made, subcultured once more in bovine kidney cells and, from the latter, propagated through 26 serial transfers in HeLa. All but 3 of these latter passages, to be commented on below, gave a similar picture: the great majority of the cells rounded up in large clumps 24 hours after inoculation with undiluted tissue culture fluid, and the cultures sloughed off the glass by the 2nd or 3rd day.

In Chart 1, HeLa cell cultures, diagrammed above the dotted line, were renewed with Eagle's basal medium containing chick serum, whereas those below were renewed with Ginsberg's fluid. This change was made more for convenience than for any specific reason, and no differences were observed in the alternating HeLa passages. Later, however, in the course of serial HeLa transfers, the 16th passage was inadvertently inoculated into cultures renewed with Eagle's basal medium containing 5% normal cow serum, and the cellular changes were slower; the cultures were not ready for harvest before the 4th day. The 18th passage, with the same renewal fluid, was still slower. It was incomplete even on the 7th day. The 19th passage showed practically no change during the observation period. This trend was promptly reversed by changing back to Ginsberg's fluid, and the virus was readily recovered from the 16th and 18th passages.

Titers of virus at various HeLa passage levels in HeLa and bovine kidney cells are shown in Table I. Whereas in the latter cells the titers are comparable to those of bovine kidney grown virus, they are lower in the former and indicate a lack of correlation between HeLa and bovine kidney titers.

*Identification of HeLa grown IBR virus*: Two different methods were employed: 1) direct serum-neutralization in HeLa and bovine kidney cells and 2) calf inoculation.

1. Results of serum-neutralization tests on various passage levels of HeLa grown virus (Table II) are unequivocal in bovine kidney cells. Although virus titers in HeLa cultures, in the presence of normal guinea-pig serum, seem to be somewhat lower than expected, the log neutralization indices are sufficiently important for a definite identification of the HeLa grown agent as IBR virus.

2. Sixteen normal calves were bled, divided into 4 groups of 4 and inoculated as follows: animals of the first group each received 2 ml undiluted fluid ($10^{7.5}$TC-$ID_{50}$) of the 2nd

TABLE III. Febrile Response and Symptoms in Calves Inoculated with 2nd Bovine Kidney or 10th HeLa Passage IBR Virus Either by Intramuscular or Intranasal Routes.

| Inoculum and route of admin. | Febrile response: Post 1st inoc. | Febrile response: Post challenge | Symptoms and duration: Activity | Appetite | Feces | Respiration | Nasal discharge‡ | Watery eyes |
|---|---|---|---|---|---|---|---|---|
| BK-2* | + 4 | — | N | N | N | N | — | — |
| 2 ml U.T.C.F.† | — | — | N | N | N | N | — | — |
| IM | + 2 | — | N | N | N | N | — | — |
| ($10^{7.5}$ TC-$ID_{50}$) | — | — | N | N | N | N | — | — |
| HeLa-10 | — | — | N | N | N | N | — | — |
| 2 ml U.T.C.F. | — | — | N | N | N | N | — | — |
| IM | — | — | N | N | N | N | — | — |
| ($10^{6.2}$ TC-$ID_{50}$) | — | — | N | N | N | N | — | — |
| BK-2 | +11 | — | I-10 | NA-4, R-6 | H-3 | N | +11 | + 9 |
| 4 ml U.T.C.F. | — | — | N | N | N | N | — | + 2 |
| IN | +10 | — | I-10 | NA-5, R-6 | H-2 | L-6 | + 9 | + 6 |
| ($10^{7.8}$ TC-$ID_{50}$) | + 8 | — | I- 9 | NA-3, R-8 | N | N | +10 | + 8 |
| HeLa-10 | + 2 | — | N | N | N | N | — | — |
| 4 ml U.T.C.F. | — | — | N | N | N | N | — | — |
| IN | + 5 | — | I- 2 | R-2 | N | N | — | — |
| ($10^{6.5}$ TC-$ID_{50}$) | +17 | +11 | I-37 | NA-5, R-32 | H-2 | L-7 | +15 | +10 |
| Challenge controls§ | | + 6 | I- 8 | NA-6, R-3 | D-5 | L-4 | + 5 | + 1 |
| BK-2 | | +12 | I-10 | NA-6, R-4 | D-4 | N | + 4 | — |
| 4 ml U.T.C.F. | | + 7 | I- 7 | NA-5, R-3 | N | N | — | — |
| IN | | + 7 | I- 9 | NA-4, R-6 | N | N | + 5 | — |

* BK-2 = 2nd passage in bovine kidney culture. † U.T.C.F. = Undiluted tissue culture fluid. ‡ — = Fever or symptoms not present; + and digit = Fever of *103.5°F or higher* or symptoms present and No. of days. § Inoculated when the four other groups were challenged at 4 wk.
N = Normal; NA = No appetite; I-10 = Inactive for 10 days.
R = Reduced appetite; L = Labored; D = Diarrhea; IM = Intramuscularly; IN = Intranasal spray and H = Hard.

clear explanation for the apparent lack of correlation between titers of HeLa grown virus in HeLa and bovine cells save, perhaps, for a generally lesser sensitivity of HeLa cells, which may vary from one lot of culture to another. In this connection, it was of interest to note that, after being established, serial propagation of the virus in HeLa cells was almost interrupted by changing to a renewal fluid containing 5% cow serum. This incident illustrates the importance of the renewal fluid when propagating virus in tissue culture, especially taking into consideration the fact that the cow serum used in the medium contained no detectable IBR neutralizing antibodies. It may also explain the relatively low virus titers, (Table II), obtained in HeLa cultures in the presence of normal guinea-pig serum, at a final concentration of 5%. It may be that use of Ginsberg's fluid at the initiation of this study might have accelerated adaptation of IBR virus to the HeLa cell.

With regard to the milder reaction of susceptible calves to HeLa-grown IBR virus in-

TABLE IV. Serum-Neutralization Indices of Calves Inoculated with 2nd Bovine Kidney or 10th HeLa Passage IBR Virus by Either Intramuscular or Intranasal Routes.

| Inoculum and route of admin.† | Log neutralization index: Weeks after inoc. 0 | 2 | 4* | 6 |
|---|---|---|---|---|
| BK-2 | $10^0$ | $10^{4.0}$ | $10^{3.5}$ | $>10^{4.5}$ |
| 2 ml U.T.C.F. | " | $>10^{4.5}$ | $>10^{4.5}$ | " |
| IM | " | $10^{4.0}$ | $10^{3.0}$ | " |
| ($10^{7.5}$ TC-$ID_{50}$) | " | $10^{3.5}$ | " | " |
| HeLa-10 | $>10^3$ | $>10^{4.5}$ | $>10^{4.5}$ | " |
| 2 ml U.T.C.F. | $10^0$ | " | $10^{3.5}$ | " |
| IM | " | " | " | " |
| ($10^{6.2}$ TC-$ID_{50}$) | " | $10^{3.5}$ | " | " |
| BK-2 | " | $>10^{4.5}$ | $>10^{4.5}$ | " |
| 4 ml U.T.C.F. | $>10^{3.5}$ | " | " | " |
| IN | $10^0$ | " | " | " |
| ($10^{7.8}$ TC-$ID_{50}$) | " | " | " | " |
| HeLa-10 | $10^{0.5}$ | " | " | " |
| 4 ml U.T.C.F. | " | " | " | " |
| IN | $10^0$ | " | " | " |
| ($10^{6.5}$ TC-$ID_{50}$) | " | $10^{4.0}$ | " | " |
| None | | | $10^0$ | " |
| (challenge controls) | | | " | " |
| | | | " | " |
| | | | " | " |

* Day of challenge.
† See Table III for explanation of symbols.

tranasally, it is doubtful that this was due to the relatively lower, but considerable, virus dosage the animals received. It suggests rather some modification of the virulence of the virus to the species by its serial propagation in a foreign host. One point of concern was the fact that 2 of 20 animals, originating from premises supposedly free of IBR, were found to have appreciable levels of neutralizing antibodies.

In view of its rapid destructive action once adapted to HeLa cells, one cannot help but speculate on what the effect of IBR virus could be on other human cancer cells *in vitro* or *in vivo*.

*Summary.* Following an essentially alternating passage method between HeLa and bovine embryo kidney cultures, infectious bovine rhinotracheitis (IBR) virus was adapted to HeLa cells and propagated through 26 serial transfers. The adapted virus rapidly destroyed HeLa cells and yielded, when measured in bovine cultures, virus titers comparable to those of bovine kidney-grown virus. It was specifically neutralized by an immune guinea-pig serum prepared against bovine-grown virus and, although of somewhat lesser virulence to calves than the parent virus, it induced a comparable level of neutralizing antibodies in all animals and afforded them a solid protection against a virulent challenge.

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## Oxidase Activity in Blood Serum from Different Species. (23256)

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During a study of demyelinating disease in lambs from ewes feeding on seaweeds a question regarding distribution of serum copper in the ewes arose. This disease in lambs resembles swayback as it is known in Great Britain and is accompanied by low blood copper in the ewes(1). Since human serum contains a copper containing protein, ceruloplasmin, with oxidase activity, it was considered interesting in this connection to learn about a corresponding oxidase activity in sera from sheep. According to Bearn and Kunkel(2) all oxidase activity of human blood serum is confined to ceruloplasmin. Therefore oxidase activity of unfractionated blood sera from a few species towards para-phenylenediamine was determined, to investigate relative content of ceruloplasmin in these sera.

*Method.* The method employed was the same as used by Ravin(3). Each sample for determination was a buffered solution containing 0.1 ml serum and 1 ml 0.5% p-phenylenediamine in a total volume of 10.1 ml. The optical density of this solution for 1 cm light path was determined in a Beckman Spectrophotometer at 530 mu after incubating 1 hour at 37°C. Each sample was tested with and without Sodium Azide and the difference in optical density calculated.

*Results.* The results are presented in Table I.

Average oxidase activity in the tested human sera is thus significantly higher than in sera from the other tested species.