1. Wallraff, E. B., Snow, I. B., J. Immunol., 1964, v93, 625.

2. Huppert, M., Bailey, J., Sabouraudia, 1963, v2, 284.

3. Wallraff, E. B., Wilson, S., Amer. Rev. Res. Dis., 1965, in press.

4. Pappagianis, D., Smith, C. E., Kobayashi, G. S., Saito, M. T., J. Infect. Dis., 1961, v108, 35.

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Physical and Chemical Properties of H-1 Virus. II. Partial Purification.* (30336)

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H-1 virus is one of a group of small agents that produces a specific deformity when they are injected into newborn hamsters(1,2). A previous paper(3) noted that the hemagglutinating property of this virus was stable over a wide range of heat and pH changes. It was observed during these studies, which were done with virus prepared from filtrates of infected hamster livers, that precipitates formed at pH 4. This finding is the basis for the method used for partial purification of H-1 virus described in this paper.

Methods and materials. Virus. Two sources of H-1 virus were used for these experiments: 1) H-1 (ham) was derived from livers of baby hamsters injected 5 days previously with H-1 virus that had had 6 passages in baby hamsters after its original isolation from the transplantable human tumor HEp #1(1, 4). This material was prepared as a 10% homogenate of pooled livers in distilled water and then filtered through a 0.03 Selas filter. It was kept at 4°C until used. 2) H-1 (tc) was also originally derived from HEp #1, but was passed in newborn hamsters for 8 passages and then grown in a tissue culture of Salk monkey heart for 13 days by Dr. H. W. Toolan.

Virus hemagglutination activity was assayed by hemagglutination of guinea pig red blood cells and reported in hemagglutination (HA) units as the reciprocal of the highest dilution that completely agglutinated all the red cells.

Virus infectivity studies employed newborn hamsters injected within 48 hours after birth. Virus was detected in these animals by death, deformity or the production of antibodies, depending on whether large or small amounts of infectious virus were present.

Anti H-1 globulin was prepared by combining equal amounts of cold saturated ammonium sulfate and serum from adult hamsters injected at birth and deformed by the H-1 virus. The resulting precipitate was dissolved in distilled water, reprecipitated with saturated ammonium sulfate and dialyzed overnight against saline.

Protein was determined by the Folin-Ciocolteau method(5).

Ultracentrifugation was performed in a Beckman-Spinco model L preparative ultracentrifuge using either a 40 rotor or a SW-39, as noted.

Electron microscope examinations. A small drop of suspension of the isolated fraction was placed on the carbon film on a 400-mesh grid. Before the thin layer of fluid remaining on the grid had dried, a drop of 2% phosphotungstic acid, adjusted to pH 7.0 with NaOH, was applied. Pictures were taken with a JEM-7 electron microscope (high tension 80 KV, double condensor, 50 μ objective aperture, initial magnifications $\times$ 40,000 and $\times$ 80,000).

Results. Table I shows some typical data. As had been noted previously, when the H-1

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TABLE I. Results of Partial Purification of H-1 Virus.

Sample	Protein*	HA†
H-1 (ham) pool‡	50,000	4,000
pH 4 super	42,000	0
pH 10	7,600	3,200
Butanol-extracted	896	2,460
H-1 (tc)	154,400	25,600
pH 4 super	156,000	850
pH 10	1,750	6,300
Butanol-extracted	850	12,800

* Total gamma of protein.
† Total HA units.
‡ "Pool" = filtrate of 10% homogenate of pooled livers from baby hamsters injected 5 days previously with H-1 virus. When this pool was adjusted to pH 4 with 0.1 N HCl, a precipitate formed which was recovered by centrifugation at 2400 rpm. The "pH 4 super" was tested as noted on the Table while the precipitate was dissolved in "pH 10 buffer," which was also tested, and then extracted with an equal volume of n-butanol. After centrifugation and dialysis to remove the butanol, the final product was tested as "butanol-extracted."

virus preparations were adjusted to pH 4 with 0.1 N HCl, a precipitate formed which could be recovered by centrifugation at 2400 rpm for 15 minutes. These precipitates, which contained almost all the residual viral HA activity, were soluble in pH 10 buffer with very little loss of activity. Such solutions were extracted with an equal volume of n-butanol by shaking at room temperature and then centrifuging at 2400 rpm for 15 minutes to separate the phases. The lower aqueous phase was dialyzed overnight at room temperature to remove the butanol. The final product contained at least 50% of the original viral HA activity but only 2% and 0.6% of the initial protein in H-1 (ham) and H-1 (tc), respectively.

The dialyzed butanol-extracted viral preparation was then further treated by centrifugation. Two methods were used: (1) centrifugation at 40,000 rpm in a 40 rotor for 5 hours; and (2) density gradient centrifugation at 35,000 rpm in a SW-39 rotor for 16 hours, with 37% cesium chloride as the gradient-forming substance. In the former method the supernatant fluid and bottom 0.5 ml were collected. All the viral HA activity but only 20% of the protein was present in the lowest fraction (Table II). Fig. 1A is an electron photomicrograph of purified virus to show concentration achieved by this technique. The inset is a magnification of individual particles. In the gradient technique, 1 ml fractions were collected in sequence from the top and dialyzed against distilled water. Fig. 2 shows the results of assays of fractions for HA. Two bands of HA activity were seen, one at a density of approximately 1.3 (light) and the other 1.4 (heavy). Both zones had similar HA titers but infectivity was 4 logs greater in the heavier band. The electron photomicrograph showed a concentration of virus in the heavy zone but little in the lighter which, however, contained many smaller particles (Fig. 1D). When samples of both the 1.4 and 1.3 bands were mixed with anti-H-1 globulin, the intact virus particles of the heavy zone were aggregated (Fig. 1B and C) and the material of the lighter zone clumped. The fragments seen in this latter band were interpreted as agglutinated sub-units of the virus protein coats.

Discussion. Precipitation at pH 4, solution at pH 10 and extraction with butanol have been used for purification of poliovirus with considerable success(6). Breese *et al*(7) have used sonication combined with chloroform-butanol extraction to purify RV(8), an agent closely related to the H-viruses. H-1 virus, which resembles poliovirus in its stability, appears to act in the same manner as the latter virus to similar purification procedures. The method described here resulted in a product in which more than 99% of the initial protein was removed but at least 50% of the original viral HA activity was retained. Previous work(9) has shown that H-1 virus is also completely resistant to treatment with

TABLE II. Results of Centrifugation* of Butanol-Extracted H-1 (ham).

Sample	Protein†	HA‡
Butanol-extracted§	896	2,460
Upper	788	0
Lower	183	2,000+

* 40,000 rpm for 5 hr.
† Total gamma of protein.
‡ Total HA units.
§ After centrifugation of butanol-extracted H-1 virus, the "lower" 0.5 ml in the tube contained only 20% of the protein of the original but all the HA activity. The remaining supernatant fluid ("upper") had no HA activity.

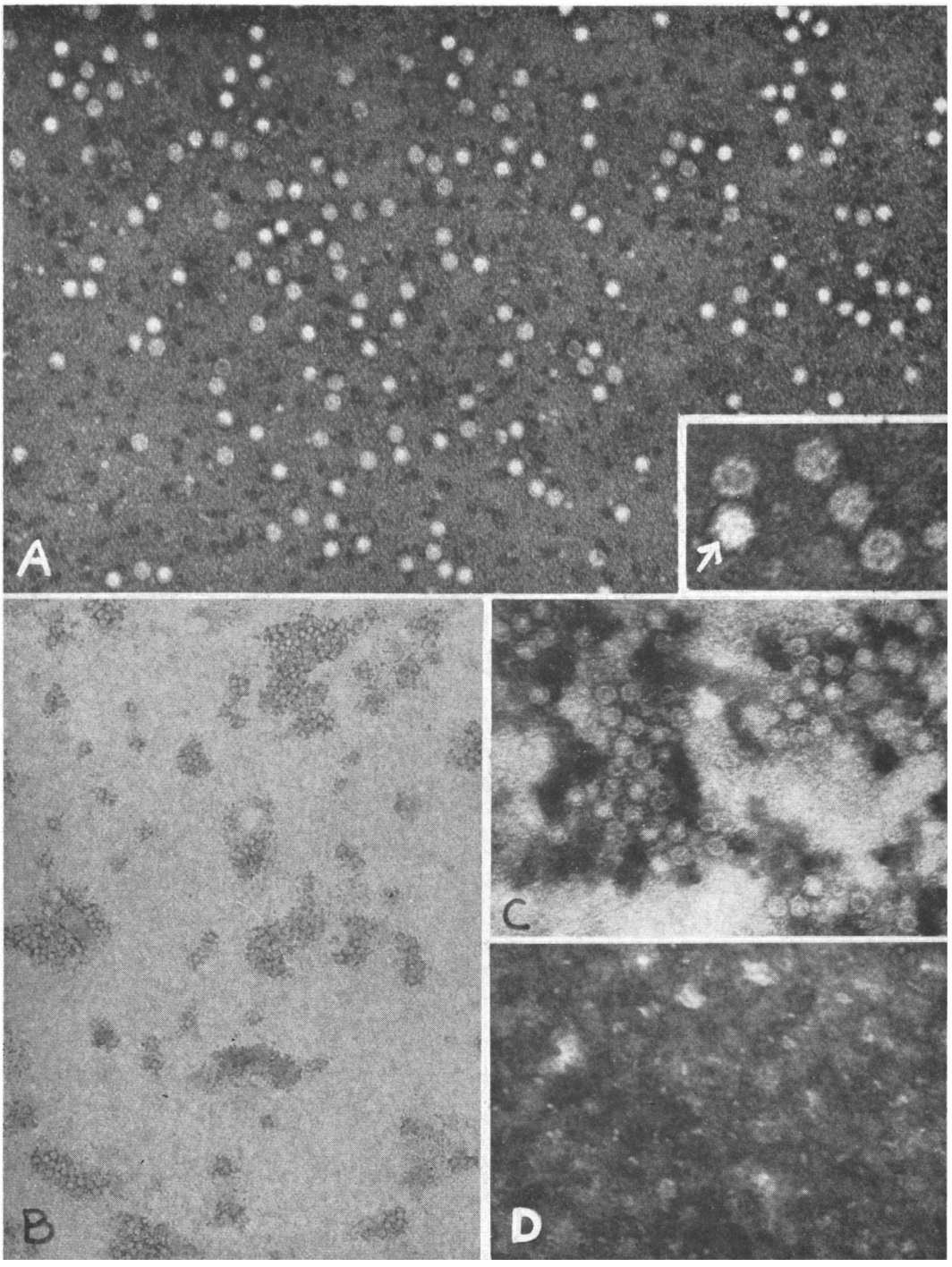

FIG. 1. *Electron micrographs of fractions of H-1 (ham).* A: Material from bottom 0.5 ml of tube of butanol-extracted preparation centrifuged at 40,000 rpm for 5 hr (× 100,000). Inset shows virus particles at higher magnification. Areas can be noted (arrow) which appear to be capsomere elements of the protein coat (× 300,000). B: Heavy (1.4) band of density gradient plus anti-H-1 globulin. Aggregated virus particles are seen (× 40,000). C: Same material as B magnified. Some aggregated "empty" particles are present (× 120,000). D: Material from light (1.3) band showing numerous small units, 40 Å in diameter (× 120,000).

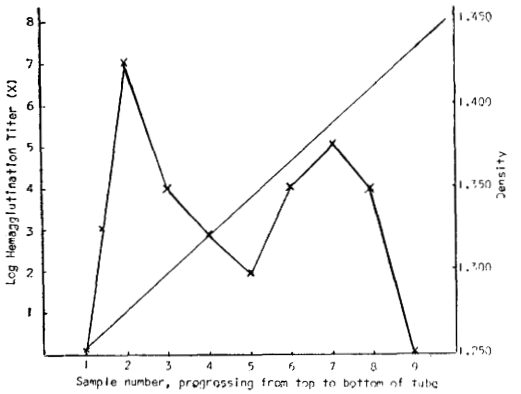

FIG. 2. Equilibrium density gradient ultracentrifugation of H-1 (ham) with cesium chloride: HA titer of fractions.

ribonuclease, deoxyribonuclease, trypsin, chymotrypsin and pepsin. It should be possible, therefore, to obtain quantities of highly purified H-1 virus, suitable for refined chemical and physical studies, if chemical and enzymatic treatment are combined with ultracentrifugal techniques.

The density gradient studies of these experiments confirmed the presence of 2 bands of HA activity as was previously noted for H-1 virus(9), and the related RV(7) and X-14(10). Intact virus appears to have a density of approximately 1.4. The lighter fraction, with a density of approximately 1.3, has HA activity but little infectivity. The present electron microscope studies indicated that this lighter band contained few complete virus particles. Breese *et al*(7) reported a lighter fraction for RV containing 80 Å particles made up of 20 Å subunits. This agreed with the observations of Vasquez *et al*(11) who noted that RV has capsomere elements about 20 by 30 Å. The clumped material for the lighter zone reported herein was similar in size to structures noted in highly magnified H-1 virus particles (Fig. 1A, inset). They measured approximately 40 Å in diameter and were suggestive of capsomere subunits of the viral protein coats.

Present studies indicate that H-1 virus is a spherical agent 230 ± 20 Å in diameter. This size range was noted for both sources of H-1 virus, namely H-1 (ham) and H-1 (tc), and agrees generally with previous estimates of this agent(9,12,13). H-1 is thus apparently larger than RV, which has been described as being 180 ± 15 Å(7,11) but similar to X-14, measured as 220 ± 20 Å(10). If the material seen in the lighter band (Fig. 1B) has been interpreted correctly as protein coat subunits, then they too are larger than those reported for RV.

H-1, X-14 and the other H-viruses such as H-3 and H-B(14), form a group of small agents that have a similar biological activity in the newborn hamster. They fall into 3 types serologically (H-1, H-B, and H-3) but can be individually distinguished on the basis of patterns of agglutination of red blood cells from different species of animals(14,15). RV and X-14 are antigenically related to H-3 while H-1 and H-B are each antigenically distinct. The nucleic acid of any agent in this group has not been determined, though indirect evidence indicates that it may be of the DNA type(16,17,18,19).

Summary. Partially purified H-1 virus was prepared by precipitation at pH 4, solution at pH 10 and extraction with butanol, followed by ultracentrifugation.

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1. Toolan, H. W., Science, 1960, v131, 1446.
2. Kilham, L., Virology, 1961, v13, 141.
3. Greene, E. L., Proc. Soc. Exp. Biol. and Med., 1965, v118, 973.
4. Toolan, H. W., Dalldorf, G., Barclay, M., Chandra, S., Moore, A. E., Proc Nat. Acad. Sci., 1960, v46, 1256.
5. Kabat, E. A., Mayer, M. M., Experimental Immunochemistry, 2nd ed., Charles C Thomas, Springfield, Ill., 1961, 556.
6. Schwerdt, C. E., Schaffer, F. L., Ann. N. Y. Acad. Sci., 1955, v61, 740.
7. Breese, S. S., Howatson, A. F., Chany, C., Virology, 1964, v24, 598.
8. Kilham, L., Olivier, L. J., ibid., 1959, v7, 428.
9. Greene, E. L., Thesis, (SKI-Cornell), 1964.
10. Payne, F. E., Beals, T. F., Preston, R. E., Virology, 1964, v23, 109.
11. Vasquez, C., Brailovsky, C., Exp. Mol. Path., 1965, v4, 130.
12. Chandra, S., Toolan, H. W., J. Nat. Cancer Inst., 1961, v27, 1405.
13. Toolan, H. W., Saunders, E. L., Greene, E. L.,

Fabrizio, D. P. A., Virology, 1964, v22, 286.
14. Toolan, H. W., Proc. Am. Assn. Cancer Res., 1964, v5, 64.
15. Moore, A. E., Virology, 1962, v18, 182.
16. Dawe, C. J., Kilham. L., Morgan, W. D., J. Nat. Cancer Inst., 1961, v27, 221.
17. Cochran, K. W., Payne, F. E., Proc. Soc. Exp. Biol. and Med., 1964, v115, 471.
18. Hampton, E. G., Cancer Res., 1964, v24, 1534.
19. Whalley, J. M., Biochem. J., 1965, v94, 10.

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Reduction in Life Expectancy as a Measure of Radiation-Induced Genetic Damage in Mice.* (30337)

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A general assumption concerning the genetic consequence of exposure to ionizing radiation is that most mutations are deleterious and will in one way or another affect the fitness of an organism. One logical effect of a genetic decrement in fitness could be a reduction in life span through early mortality. The Report of the United Nations Scientific Committee on the Effects of Atomic Radiation(1) states than an increase in mutation rates can be expected to give rise to a substantial reduction in viability even though the mutations produced are not responsible for visible harmful traits. The report also states, "It has, in fact, been shown in mice that the offspring of irradiated parents have a higher mortality than control animals during the early part of life." The hope was expressed (1) that more work would be done in this field of endeavor. The following is a report on one of a series of life span studies being done at this Laboratory.

Experimental methods. One sib pair of RFM strain mice from a line with a history of more than 25 generations of inbreeding propagated the mice used in this study. The experimental approach used to obtain mice for comparative life span studies is illustrated in Fig. 1. Two sib pair littermates from the parent pair were used to start 2 lines. Male mice used to propagate the irradiated line were given whole body exposures of 200 rads of X-rays at 25 $\pm$ 2 days of age and were caged with their sisters. The germ cells used for breeding had reached the spermatid or earlier stages of maturation at time of exposure. The mean time-interval between X-irradiation of irradiated males used to propagate the irradiated line and productive breeding was 27 days. Irradiated line mice reported here were unirradiated second litter male and female offspring from 20 generations of X-irradiated sires (F^{I}_{21}) and their second litter virgin daughters (F^{I}_{22}).

Following 10 generations of X-irradiation, a line designated as the irradiated sub-line (Fig. 1) was started. This line has been continued with no further X-ray exposure. Irradiated sub-line mice reported here were unirradiated second litter male and female offspring from 10 generations of X-irradiated males followed by 10 generations with no X-ray exposure (F^{Is}_{21}) and their second litter virgin daughters (F^{Is}_{22}).

Control line mice were second litter male and female offspring from 20 generations of sib-mated unirradiated males (F^{C}_{21}) and their second litter virgin daughters (F^{C}_{22}).

The following X-ray factors were used to provide X-irradiation to males in the experimental lines: 250 KVP, 30 ma, Thoreaus II filter, 2.6 mm Cu HVL, 60 cm target-to-specimen distance, and 50 rads/minute dose rate.

Results and discussion. Mean life span for the 9 groups of mice in question is tabulated in Table I. Breeding males of this strain have a longer life expectancy than do breed-

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