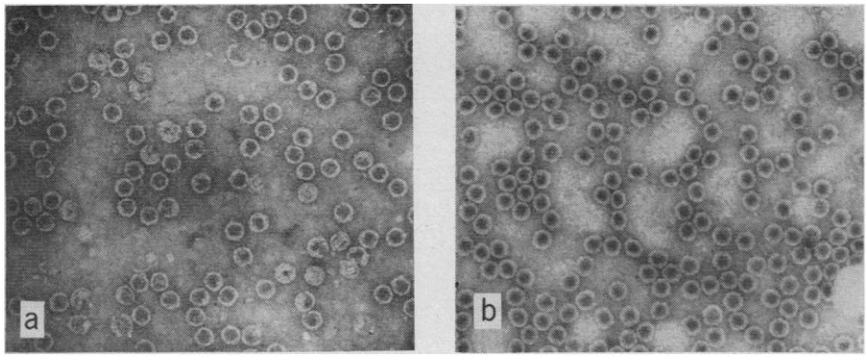

Fig. 2. Electron micrographs negatively contrasted with uranyl oxalate. (a) Reconstituted incubation mixture. (b) Bacteriophage f_2. Photographs were taken on a Philips EM-200 electron microscope at 80 kv with an anticontamination device, double-condenser illumination, and a 35-μm objective aperture. Thin carbon films were used for specimen support.

in the assembly of a few of the 37*S* particles. Objective statistical evaluation of the extent of degradation was complicated by the fact that preservation is increased in areas of thicker stain and that relative stain thickness between two grids is difficult to evaluate. Bacteriophage f_2, "artificial top component," and 37*S* particles were also examined by electron microscopy after staining with 1 percent phosphotungstate (PTA) at *p*H 7. The latter two specimens again appeared identical and differed from the appearance of negatively contrasted f_2 only in that the PTA stain was largely excluded from the virus but extensively penetrated the empty shell structure (*3*). Particle diameters were obtained in each case by internal calibration against the 84.4-Å half-period (*6*) of admixed crystals of beef-liver catalase. "Artificial top component" and 37*S* particles both had a mean diameter of 238 ± 10 Å and f_2 measured 246 ± 7 Å; therefore they have an identical size within experimental error.

The identical morphological appearance of the 37*S* particle and "artificial top component" supports the hypothesis that both should have a similar frictional coefficient. Sedimentation velocity experiments with the photoelectric scanning optical system provided an $s_{20,w}$ value of 41*S* for "artificial top component" which contained about 3 percent RNA as estimated from the 280/260 nm absorbancy ratio. Using the physical and chemical parameters obtained by other workers as previously referred to by us (*4*), we calculated the sedimentation coefficient of f_2 protein shells to be 39*S*, which is in good agreement with that observed for the 37*S* particles.

The formation of the 37*S* particle seems compatible (within the limitations of the characterization employed) with the hypothesis that it represents the reconstitution of f_2 capsids without the intervention of RNA, core protein, or other assembly-directing influence. We have thus far obtained no indication as to whether either 11 or 5.5*S* subunits are direct intermediates in the assembly process, nor have we as yet unambiguously identified additional intermediates in the reconstitution. Consequently, detailed mechanistic proposals for capsomer interactions and assembly of 37*S* particles are premature at this time (*7*).

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8. Supported by PHS grant HE 11822 and NSF grant GB-7042. We thank Professor R. Swan and the Department of Anatomy for providing time on their Philips EM-200 electron microscope.

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Australia Antigen: Distribution during Cohn Ethanol Fractionation of Human Plasma

Abstract. *When plasma containing a hepatitis-associated antigen (Au/SH) is fractionated, the antigen is localized in fractions III and IV with none in fraction II and only small amounts in fractions I and V. The amount of antigen found in each of these fractions is probably not predictive of clinical infectivity of Cohn ethanol fractions from normal pooled plasma.*

The fractionation of human plasma by methods 6 and 9 of Cohn and associates (*1*) has been adopted on an industrial scale and yields protein fractions of therapeutic usefulness. However, the risk of transmitting serum hepatitis has been a deterrent to maximum utilization of some plasma protein fractions. With the demonstration that a specific antigen (the "Australia antigen," now often known as hepatitis-associated antigen) was often associated with hepatitis (*2*) and the subsequent confirmation and extension of this association in numerous laboratories (*3–5*), an assay became available which allowed us to follow this antigen (Au/SH) when plasma is fractionated.

For this study, we began with human plasmas containing Au/SH. These plasmas were detected during screening of plasmapheresis donors (*4*), and the Au/SH content of the Cohn fractions was assessed by means of a gel-diffusion procedure (*3*). Two 500-ml portions of different Au/SH-positive units of plasma were fractionated by Cohn methods 6 and 9 (*1*). During these fractionations, samples (0.5 g) of the moist precipitates or of the alcoholic supernatant (5 to 20 ml) were diluted with water and lyophilized to remove alcohol. The dry powders were then dissolved in a measured volume of an appropriate buffer and assayed for Au/SH content (*6*). In all cases the

samples for assay were more concentrated compared to the starting plasma. The two experiments gave comparable results and these are summarized in Table 1.

The data are expressed as the total number of units of Au/SH in each fraction derived from 500 ml of plasma. Since these units are calculated from serial dilutions (*6*), the values may vary by a factor of two from a single tube dilution difference.

These data show that the total amount of Au/SH in various fractions is widely different. For example, fraction II (normal immunoglobulin) is devoid of measurable antigen, whereas fractions and subfractions of III and IV contain appreciable amounts. This distribution is different from that reported by Pennell (*7*), in which various other viruses added to plasma were removed predominantly in fractions I and in II + III, very little being in fraction IV. Our findings do, however, corroborate recorded, long clinical experience that fraction II does not transmit hepatitis while other fractions, such as I or III, are often so implicated. The data indicate that the positive Au/SH test obtained with our fraction I may be due simply to the entrapment of some of the strongly Au/SH-positive supernatant solution. It must be emphasized that this was crude fraction I which had not been further processed to the fibrinogen product for human administration. When this is done, a substantial decrease in Au/SH content of fibrinogen is observed which can be accounted for by the amount of antigen removed in the wash solutions. We should point out that no Au/SH can be detected by the techniques used in fibrinogen prepared from normal commercial plasma pools, although in such a product the presence of hepatitis virus is assumed as a definite hazard. Similarly, the amount of Au/SH in factor IX complex (*8*) prepared from Au/SH-containing plasma is very low (Table 1). When prepared from normal plasma pools, this fraction has no Au/SH measurable by the technique used, but, nonetheless, may be associated with transmission of hepatitis.

The Au/SH precipitated with fractions II + III was clearly localized in fraction III. When this crude fraction III was further separated into fraction III-1 (isoagglutinins) and fraction III-2,3 (prothrombin, plasminogen) (*1*), essentially all of the Au/SH was found in fraction III-1. Fraction II contained no detectable Au/SH. We have examined this fraction for antibody to Au/SH by both agar gel diffusion (*3*) and complement fixation (*9*) assays. No specific antibody could be detected.

Table 1. The distribution of Au/SH in Cohn ethanol fractions prepared from Au/SH-containing plasma. Results are expressed as Au/SH units in each fraction derived from 500 ml of plasma. Where two values appear for the same item, there were two determinations from the same sample.

Fraction	Au/SH (unit/fraction) (*6*)			
	Fractionation I		Fractionation II	
Plasma	400,000		400,000	
Cryoprecipitate (*12*)	5,000		1,250	2,000
Fraction I	2,750		2,500	
Supernatant I	490,000	367,000	224,000	
Factor IX complex (*8*)	66		10	
Supernatant I*	477,000	358,000	222,000	
Fraction II + III	33,000		Negative	7,700
Supernatant II + III	256,000		354,000	384,000
Supernatant IV-1	187,500	134,000	150,000	96,000
Fraction IV-1	56,000		87,600	
Supernatant IV-4	65,200		Negative	
Fraction IV-4	22,000	27,000	34,000	
Supernatant V	Negative		Negative	
Fraction V	20,000	23,000	Negative	
Supernatant II + III W	Negative (*3*)		Negative	
Fraction II + III W	50,000		17,000	33,000
Supernatant III	Negative		Negative	
Fraction III	36,400			
Fraction III-1			28,800	
Fraction III-2,3			200	
Supernatant II	Negative		Negative	
Fraction II	Negative		Negative	

* After removal of factor IX complex.

Most of the Au/SH remains in solution during the precipitation of fractions II + III. The distribution of this Au/SH is somewhat unclear, but it appears that fraction IV-1 contains several times as much Au/SH as fraction IV-4 does. Our direct observation of Au/SH in fraction IV is in accord with an earlier report of the transmission of hepatitis by this fraction (*10*). Fraction V (crude albumin) contained much less Au/SH than fraction IV did. When a sample of this albumin containing Au/SH was heated at 60°C for 10 hours in the presence of stabilizers (*11*), the Au/SH precipitin reaction of this sample was not destroyed or diminished. This indicates that the Au/SH antigen assay cannot be equated with infectivity, since heat-treated albumin is known not to transmit serum hepatitis (*11*). Thus, since the antigenicity of Au/SH is what we have measured in these migration studies and since antigenicity and infectivity cannot be equated, we conclude that the amount of Au/SH in each of these fractions is probably not predictive of clinical infectivity of plasma fractions from normal pooled plasma.

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is, 16 × 50 or 800 units of Au/SH per milliliter. Each 500 ml of plasma thus contained 400,000 units of Au/SH. The total number of units of Au/SH in each fraction was then calculated relative to the total number of units in the starting plasma (Table 1).

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Reproductive System of Hutchinsoniella macracantha

Abstract. *The cephalocarid crustacean* Hutchinsoniella macracantha *is a hermaphroditic species. Ova and sperm develop simultaneously. Ovaries and testes are separate, but the oviducts and vasa deferentia join and exit through a pair of common genital ducts.*

The subclass Cephalocarida has been given considerable attention with regard to questions of crustacean phylogeny. Comparative studies of external morphology, skeletomusculature, larval development, and behavior in the best-known species, *Hutchinsoniella macracantha* Sanders (*1*), all demonstrate that members of this subclass are the most primitive known crustaceans (*2*). These studies also suggest some of the basic patterns in crustacean evolution. Our investigation of the internal anatomy reveals a condition of the reproductive system which, while not necessarily primitive, is certainly unusual among arthropods (*3*).

Hutchinsoniella macracantha is a true hermaphrodite (Fig. 1). All adults possess well-developed ovaries and testes. These two pairs of organs are the only places in the genital system where chromosomal figures of dividing cells have been observed. Testes are sausage-shaped bodies located dorsal to the gut from the seventh to the twelfth postcephalic segments. Each ovary is a much smaller cluster of cells appressed to the medial surface of the tubule of the maxillary gland in the posterior portion of the cephalon.

Each oviduct arises from the ovary's medial surface and extends toward the gut within the diffuse ventrolateral mesentery that invests the ventral longitudinal muscle bundle and the gut. It then turns posteriorly and runs the length of the thorax within the mesentery and dorsal to the ventral longitudinal muscle. In the thorax, ova are sparsely distributed and small, appearing as they do within the ovary. The oviduct continues posteriorly into the abdomen, where ova begin to enlarge. In the 18th postcephalic segment the oviduct doubles back upon itself dorsally and extends forward into the thorax up to the sixth postcephalic segment. At the posterior flexure there is an accumulation of ova; here they are very large but still lack yolk. Between the flexure and the terminal portion of the oviduct there is one more ovum; it is extremely large, extends through several segments, and is heavily laden with yolk. This is the mature ovum, ready to be laid. The maturation of but a single pair of ova relates to the fact that cephalocarids brood only one pair of eggs at a time.

The anterior ends of the pair of testes are joined together in the seventh postcephalic segment, dorsal to the gut. From this juncture spring the vasa deferentia, each of which descends obliquely anteriorly and joins the oviduct in the sixth postcephalic segment. The now common genital duct descends into the sixth thoracic limb and exits on the posterior face of the protopod.

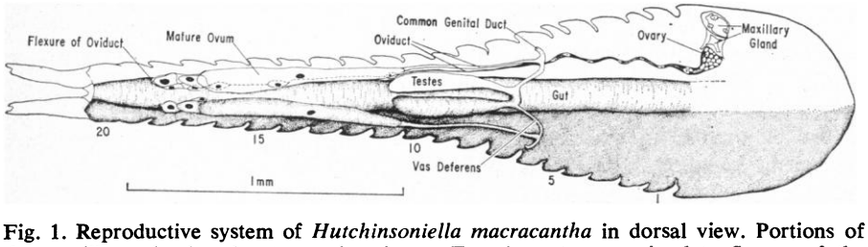

Fig. 1. Reproductive system of *Hutchinsoniella macracantha* in dorsal view. Portions of the maxillary gland and gut are also shown. Female system proximal to flexure of the oviduct is not shown on the right side of the animal. Numbers beneath the figure label postcephalic segments.

Among the free-living Crustacea are very few cases of hermaphroditism in which both sexes function simultaneously (*4*). Within the subclass Cirripedia, the Thoracica are generally cross-fertilizing hermaphrodites, and here the genital pores are on separate segments. Cephalocarids, as exemplified by *H. macracantha*, also generate sperm and ova simultaneously. They are unique in discharging these products through a common duct.

There is no clear-cut structural evidence that rules out the possibility of self-fertilization. However, cephalocarids are mobile and inhabit normal marine soft-bottom environments at reasonable population densities (*5*). Thus, there is no obvious adaptive reason why such organisms should practise self-fertilization.

Cephalocarids have never been observed mating. It has been assumed that the modified endopod of the seventh thoracic limb is a clasping organ; yet the separation of the genital pore from the ninth postcephalic segment, which bears the brooded egg, suggests that this modification may actually serve in directing the egg as it is laid to its place of attachment. Cephalocarid sperm is nonmotile (*6*), which indicates that individuals participating in cross-fertilization must come into intimate contact. There is no evidence of the formation of spermatophores.

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