sum) to white rats. Developing worms were recovered from rat intestines at daily intervals from 2 to 22 days after infection, and at less regular intervals thereafter.

The cells described here are located in a cluster in the rostellum. Their axons enter a nerve tract within the rostellum. This nerve tract is connected to nerves which lead to the lateral ganglia of the central nervous system (Fig. 1).

The bipolar cells contain abundant material which stains heavily with paraldehyde-fuchsin (Fig. 2). Anteriorly, the cells are prolonged into filaments, a characteristic of sensory cells in cestodes (*2*). This finding suggests that these cells are sensory, as has been decribed for neurosecretory cells in a parasitic nematode (*4*).

The cells undergo a cycle of secretion associated with development of the adult tapeworm. The cells can be recognized in the cysticercoid, but they are entirely devoid of fuchsinophilic material. Fuchsinophilia develops in worms 3 days after infection and rapidly becomes maximum (Fig. 2). This development is associated with an increase in the size of the cells. Granules of fuchsinophilic material first become obvious in axons of worms fixed 16 to 18 days after infection (Fig. 1). At the same time, the amount of fuchsinophilic material in the cells begins to decrease, and, by 40 days, the cells again fail to display fuchsinophilia.

It is impossible to assign a precise function to these cells on the basis of these studies. However, the release of neurosecretion as evidenced by the first appearance of fuchsinophilia in the axons is correlated quite closely with the shedding of the first proglottid, an event which occurs 16 to 17 days after infection. On the other hand, fuchsinophilia in the neurosecretory cells first appears just before the time that strobilization begins.

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28 August 1967

Colostral Immunoglobulin-A: Synthesis in vitro of T-Chain by Rabbit Mammary Gland

Abstract. *When minced mammary tissue from lactating rabbits was incubated in vitro with C^{14}-labeled lysine and isoleucine, it incorporated radioactivity into colostral immunoglobulin A. The only portion of this colostral molecule with significant labeling was T-chain, with little or no labeling of light or heavy chains. It was thus demonstrated that T-chains are synthesized by mammary gland. Because the remainder of the molecule was derived from unlabeled material, in vivo it was probably derived from serum.*

Colostrum, saliva, and certain other external secretions are rich in immunoglobulin A (γA), an immunoglobulin usually present only in low concentrations in serum. It has been established that secretory γA differs from serum γA and from other immunoglobulins in that the molecule contains, in addition to the usual light and heavy chains, a third kind of polypeptide called "T-chains." In man, the existence of an additional chain has been deduced from a series of studies with antiserums specific for it (*1–3*); in the rabbit, T-chains have been demonstrated in the light-chain fractions of reduced and alkylated colostral γA (*4*). Polyacrylamide electrophoresis of these light-chain fractions showed a rapidly migrating component in addition to the usual light-chain bands. In both man and rabbits, it seemed that much of the T-chain component was not covalently linked to the whole molecule (*3, 4*).

The difference between low concentrations of serum γA and the high concentrations found in secretions could be explained either by local synthesis or by some concentrating mechanism. Attempts to show transfer in humans of γA from serum to parotid saliva have either been unsuccessful (*1*) or have resulted in such small and inconstant amounts of transfer (*2*) that a concentrating mechanism seemed unlikely. Furthermore, Tomasi *et al.* (*1*), using immunofluorescence, have shown in the interstitial tissue of parotid glands many plasma cells containing γA.

To investigate a possible concentrating mechanism, secretory tissue containing few lymphoid cells but producing secretions with large amounts of γA is required. In that rabbit mammary gland fits these criteria and the constituent polypeptide chains of rabbit colostral γA can be identified (*4*) so readily, we wanted to determine which of the chains was formed by the gland.

A New Zealand White rabbit (2500 g) with a litter less than 8 hours old was killed by cervical fracture. Taking care to avoid adjacent lymph nodes, we excised the mammary gland. This gland was minced in Hanks's balanced salt solution, and six portions of about 100 mg were incubated in roller tubes in a medium containing C^{14}-lysine and isoleucine. One of the culture fluids and samples of each of the fractions described below were analyzed for the presence of labeled γA by immunoelectrophoresis and autoradiography. Both the incubation and analysis were performed as described by Hochwald *et al.* (*5, 7*). The remaining five culture fluids were pooled, passed through a diethylaminoethyl (DEAE) cellulose column, and eluted stepwise (*6*). Because most of the γA appeared to be in the 0.2*M* fraction (Table 1), this was dialyzed against 0.1*M* borate buffer, *p*H 8, and passed through a column of Sephadex G-200. The volume expected to contain excluded material was the only fraction that contained labeled γA and accounted for 8 percent of the radioactive material precipitable by

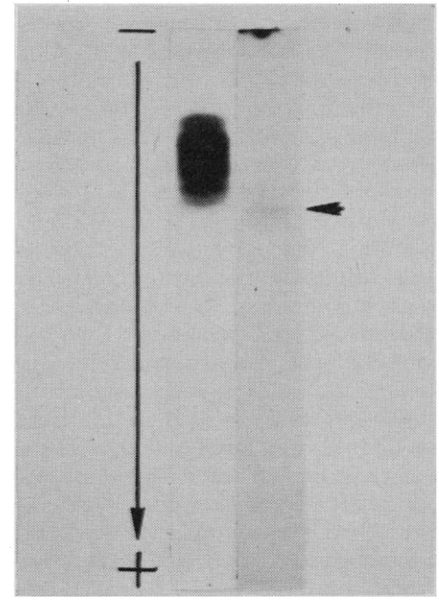

Fig. 1. Polyacrylamide electrophoresis of light chains, with autoradiograph. (Left) Dried, stained electrophoretic pattern (gel); (right) matched autoradiograph (film). A labeled band (arrow) is seen in a position anodal to the fastest light-chain component.

Table 1. DEAE-cellulose chromatography of mammary-tissue culture fluid.

NaCl* (*M*)	Recovered † (%)	Labeled γA
0	2	0
0.1	18	0
0.2	24	+++
0.3	32	+
0.5	19	0
1.0	8	0
Not recovered	7	

* In 0.005*M* phosphate buffer, *p*H 7.9. † Protein precipitated with 5 percent trichloroacetic acid. Results expressed as fraction of original TCA-insoluble radioactive protein.

trichloroacetic acid (TCA) of the original culture fluid. To this pool was added as a carrier 15 mg of nonlabeled rabbit immunoglobulin G (γG); the mixture was extensively reduced and alkylated and passed through a previously calibrated Sephadex G-200 column in the presence of guanidine. The fractions containing heavy (α and γ) and light chains had about 40 and 60 percent, respectively, of the radioactivity applied. They were dialyzed, lyophilized, and analyzed by disk electrophoresis in the presence of urea. The procedures for separation and analysis of the polypeptide chains have been described previously (*4*). The resulting patterns were typical of γG heavy and light chains. The concentration of γA was insufficient to give visible α- or T-chain bands in these patterns. After dialysis against distilled water, the stained disk gels were laid flat on a lantern slide and embedded in 2 percent agarose in saline. The embedded gels were dried under wet filter paper and exposed for 8 weeks to Kodak Royal Pan film. Spots of very dilute C^{14}-lysine were placed near the ends of the gels to serve as markers for aligning the autoradiograph.

Figure 1 shows an electrophoretic pattern of the light chains with the corresponding autoradiograph. We found a radioactive band migrating more rapidly than the light chains, with a faint trail extending into the light-chain region. Densitometric analysis of the autoradiograph showed that 75 percent of the label in the gel was in the fast component in the area where T-chain migrates. In similar analysis of the heavy-chain fractions, most of the radioactive material was retained in the spacer gel and hence seemed to be contaminating large protein.

Our data show that T-chains, which are intimately associated with colostral γA, are synthesized by mammary tissue. This observation is consistent with that of Tomasi *et al.* (*1*) who reported that T-chains are present in parotid gland acinar cells; it also extends the finding of Hochwald *et al.* (*7*) who showed that human mammary and parotid gland synthesized a protein that coprecipitated with γA. Furthermore, it seems likely that the heavy and light chains of the colostral γA are synthesized elsewhere, in that there was no significant amount of label associated with them. This latter conclusion could be invalidated if the T-chain was particularly rich in lysine or isoleucine, or both, or if there were very large pools to dilute newly synthesized heavy and light chains. Both of these possibilities seem unlikely to us. Since at least three times as much radioactivity is present in T-chains as in light chains, and since there are probably twice as many light chains as T-chains in the whole molecule, a sixfold difference in content of the two amino acids would be required to explain our data. This is not consistent either with the observed electrophoretic mobility of T-chain or with the published amino acid composition of the whole molecule (*6*). To our knowledge, there is no evidence that large pools of intracellular γA exist in secretory glands. Immunofluorescent studies of human parotid tissue (*1*) indicate that γA is formed only in plasma cells which are probably actively synthesizing the protein.

Our experiments indicate that colostrum rich in γA can be produced without local synthesis of either light or heavy chains. Therefore, it seems likely that γA is actively transported and concentrated from serum into colostrum.

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Ultrastructure of Gamma M Immunoglobulin and Alpha Macroglobulin: Electron-Microscopic Study

Abstract. *Electron microscopy of purified Waldenström macroglobulins and normal human and rabbit γM immunoglobulins revealed spider-like structures with five legs varying in length and often joining a central ring. Usually only the central more rigid part of this structure (about 150 by 170 angstroms) was clearly visible, but occasionally particles were seen with longer very flexible legs having a total span of about 350 angstroms. Molecules of γM antibody retained antibody activity during preparation for electron microscopy. Human and rabbit alpha macroglobulins revealed more rigid symmetric structures (100 by 200 angstroms) which resembled the Russian letter* ж.

The size and conformation of several serum proteins have been estimated by light scattering, x-ray diffraction, fluorescence polarization, intrinsic viscosity measurement, analytical ultracentrifugation, and electron microscopy. Some of these techniques have been applied to studies of myeloma proteins and Waldenström macroglobulins. But we are aware of no reported electron-microscopic studies of these paraproteins, and the only electron-microscopic data relating to normal γM immunoglobulins, not in complexes with antigen, have been published by Höglund and Levin (*1*) and Höglund (*2*). The limited application of electron microscopy in studies of serum macroglobulins can be partially ascribed to their apparent low electron density.

A different situation exists when the γM globulins have antibody activity and can be examined when they form complexes with an antigen of well-defined and characteristic shape. This approach has recently been utilized in electron-microscopic studies of complexes of virus and γM antibody (*2–4*). In