beled red cells were hemolyzed and passed through an appropriate Sephadex column (13) two components were obtained. One radioactive peak was associated with hemoglobin whereas the subsequent peak represented Cr^{51} not attached to hemoglobin. The relative amount of unbound chromium was variable in different species even though the tagging conditions were identical. In the rat we found approximately half of the chromium not bound to hemoglobin, whereas in the dog and especially in the human, the proportion of unattached hemoglobin was considerably smaller. When Cr^{51}-tagged hemolysate was injected into the rats a very fast plasma clearance and renal excretion of non-hemoglobin-bound Cr^{51} was observed. This was distinctly different from the clearance rate and catabolic fate of hemoglobin-bound Cr^{51}(12).

In the light of these findings, we believe that the catabolic studies with radioactive hemoglobin require very careful interpretation. Perhaps some of the existing discrepancies between human studies(14) and animal experiments could be resolved on the basis of species differences. Other apparent dissimilarities might be due to artifacts caused by the use of different radioisotope tags.

Summary. 1. The plasma clearance of I^{131}-tagged, autologous hemoglobin was studied in the rat. 2. This radioactive hemoglobin was cleared at an exponential rate, having a half-life of 25 minutes. 3. Within the range of 3 to 10.2 mg hemoglobin per 100 g body weight, clearance rate was independent of dose administered. 4. No renal elimination of hemoglobin could be demonstrated. 5. Rapid release of I^{131} from the hemoglobin was noted. This considerably increased the plasma radioactivity value over the actual hemoglobin level after only 30 minutes.

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A Toxin in the Coelomic Fluid of Scalded Starfish (*Asterias forbesi*).* (27337)

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The toxic-factor theory of the lethal effects of burns states simply the burned tissue releases a toxin which upon entering the circulatory system may induce shock and/or death. The work of Heilbrunn *et al.*(1) and Prinzmetal(2) suggested that toxins are present in a variety of vertebrate organisms. Rosenthal(3) found such toxins in the skin of rats. Simonart(4,5) reported that death, usually preceded by shock following severe scalding, is in part due to a toxic substance released at the site of injury. He found that

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TABLE I. Effect of fractionating the collection of toxic coelomic fluid. (No. of animals injected.)

	Experimental fluid		
	Fraction I collected during scalding (0–1½ min)	Fraction II collected after scalding (1½–3 min)	Control
% animals autotomized after injection of .15 cc/g	79.9 (124)	41.6 (48)	0.8 (54)

frogs could survive lethal-scald levels provided that the blood vessels of the burned area were ligated. Simonart found that the circulatory system of the scalded frog contained a toxin which has since been produced by merely heating blood *in vitro*. The author has reported a toxin in the scalded marine annelid, *Phascolosoma gouldii*, which proved lethal when injected into normal worms(6,7,8). In the present paper it is shown that a toxin appears in the coelomic fluid of burned starfish.

Materials and methods. Both male and female starfish (*Asterias forbesi*) obtained from the supply department of Marine Biological Laboratory were used. Donor animals, from which coelomic fluid was removed and later tested by injection into recipient starfish, weighed from 20 to 50 g. The donor starfish were placed in plastic bags and the animal scalded in sea water at 76°C for 1½ minutes. The coelomic fluid having escaped through the damaged arms was removed from the polyethylene bag immediately after scalding and autoclaved for 20 min at 15 lb/sq inch. The supernatant was injected intraperitoneally into recipient normal starfish of the same size in a volume of fluid proportional to weight (0.15 cc/g of starfish). Injected starfish were kept for 45 minutes in dry fingerbowls, and then returned to fingerbowls of running sea water. Since the autotomy reaction of a recipient starfish to the toxin occurred within the first 45 minutes, most experiments were terminated at that time. The toxic and control fluids injected in any one experiment were obtained by pooling coelomic fluid from 10 to 30 scalded or normal animals. In all experiments a minimum of 10 starfish was injected in each group.

In experiments designed to determine the cellular source of the toxin, the animals were dissected into arbitrary fractions, the tissues washed, suspended in test tubes of sea water, and the suspension heated for 2 minutes at 76°C. The supernatant, obtained after centrifuging, was injected into recipient starfish and its toxicity determined.

Various types of control fluids were obtained, such as coelomic fluid from normal starfish, or the fluid extruded after an animal had been injected with sea water.

Results. The most obvious reaction of starfish to experimental scalding was autotomy of the injured arm (or arms). Animals with arms scalded for 45 seconds autotomized those injured arms within 24 to 30 hours after scalding. A scald of 2 minutes induced autotomy within 8 or 9 hours.

Sixty-four starfish were scalded for 1½ minutes at 76°C, and the coelomic fluid collected from the scalded animal within 3 minutes after initiation of scalding. This donor fluid from scalded animals was injected into recipient normal starfish. Forty-six per cent of the recipients lost some or all of their arms by autotomy within 45 minutes (Fig. 1). Animals so treated were frequently found to autotomize only one or as many as all 5 arms. Only 2% of the recipient normal starfish autotomized after injection of coelomic fluid from non-scalded donor starfish.

Table I shows that the coelomic fluid collected immediately after scalding contained substantially more autotomizing toxin than fluid collected much later. Thus, coelomic fluid obtained immediately after scalding was found to produce autotomy in almost 100% of the treated (injected) animals (Table II). Table II (group 3) shows that some of the

TABLE II. A typical experiment demonstrating effect of pooled toxic coelomic fluid when assayed on 6 groups of recipient starfish. (10 animals injected per group.)

	Fluid from						
	Scalded starfish						Control starfish
Group	1	2	3	4	5	6	7
% animals autotomized after injection	100	100	80	90	90	100	0

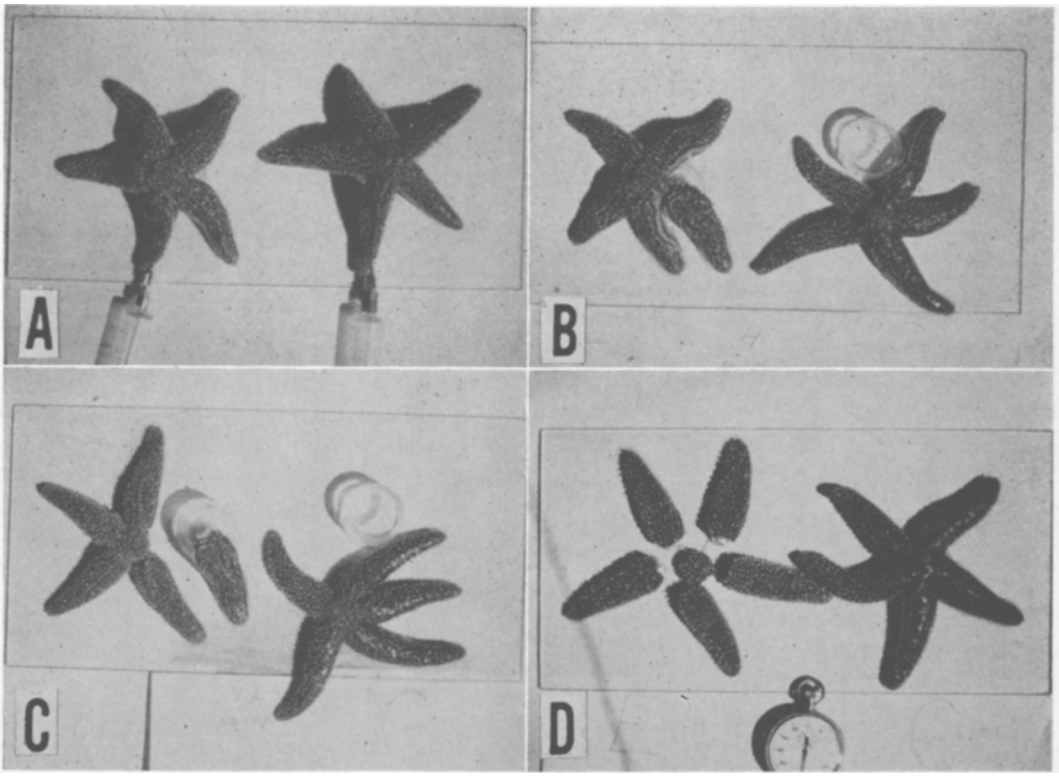

FIG. 1. Experimental (left), control (right) in each photograph. Photographs A, B, and C were taken at ½, 4, and 12 min., respectively. Onset of autotomy is seen after 4 min. in the experimental recipient in B and completed in C. Photograph D represents a second pair in which the experimental animal has autotomized all 5 arms.

animals (20%) never autotomized, and therefore might be considered "resistant" animals. It is of interest to note that when these "resistant" starfish, having survived one injection of toxic coelomic fluid, were injected several days later with a second dose of the same toxin, most of the animals survived. Attempts to demonstrate an "anti-toxic" effect in the coelomic fluid of such "resistant" starfish proved unsuccessful in *in vitro* experiments in which the coelomic fluid from "resistant" animals was incubated with toxin.

Different tissues of normal starfish were suspended in sea water and heated *in vitro* (2 min at 76°C) to determine the cellular origin of the toxin. Neither coelomic fluid nor radial nerves produced any detectable toxin upon heating. However, the supernatant fluid from heated hepatic tissue produced autotomy when injected into normal animals. Aboral tissue of the arms also gave rise to toxin. This toxin produced *in vitro* had the same physical characteristics as the toxin obtained from starfish scalded *in vivo*. Since all of the above fractions contain, in part, epithelial tissue, suspensions of epithelium isolated from the wall of the coelom was removed, suspended in sea water, and heated *in vitro*. The supernatant fluid induced autotomy when injected into normal starfish, indicating that these cells were the source of a high concentration of a toxin having the same characteristics as the burn toxin.

The toxin extracted from scalded animals was found to be a stable substance in that it retained its activity when autoclaved and/or frozen for over a year. The biological activity of the extract was lost when dialyzed against running sea water (approximately 21°C). Although the pH of toxic fluid (5.4) was lower than that of control fluid (7.2), autotomy still occurred when the pH of the toxic fluid was adjusted to pH 7.2.

The coelomic fluid of normal and scalded

TABLE III. Experiment demonstrating the lack of species specificity when starfish (*A. forbesi*) toxin (or control) is injected (0.15 cc/g) into various marine organisms. (+ indicates lethal reaction from toxin injection.)

Recipient	Donor: Starfish toxic fluid	Donor: Starfish control fluid
Starfish *(Asterias vulgarus)*	+	—
Red sea star *(Henricia sanguinolenta)*	—	—
Brittle star *(Ophioderma brevispina)*	+	—
Sea urchin *(Arbacia punctulata)*	+	—
Sand dollar *(Echinarachnius parma)*	+	—
Sea cucumber *(Thyone briareus)*	—	—
Marine annelid *(Phascolosoma gouldii)*	+	—
Horseshoe crab *(Limulus polyphemus)*	+	—

starfish was injected into various marine organisms representing 3 different phyla (Table III). Survival time varied from several hours to almost 3 days. Of the 8 species tested, 6 were susceptible to the coelomic fluid from scalded starfish.

Discussion. According to the toxic-factor theory of burns, the circulating fluid of a scalded animal should contain a toxin(s) which is partly responsible for the death of the animal. Although those experiments in which starfish autotomized after having been subjected to various scalding times may not suggest the presence of a toxin, they do indicate the point that autotomy occurs soon after scalding. The fact that coelomic fluid from scalded starfish is capable of inducing the reaction of autotomy in normal starfish, whereas coelomic fluid from non-scalded starfish has no apparent effect under otherwise identical conditions, demonstrates the presence of a factor, possibly a toxin, in the body fluid of starfish as a result of scalding.

It is interesting to note the lack of species specificity exhibited by the toxin taken from the coelomic fluid of scalded starfish, which proved lethal when injected into various other species. No explanation can be given, however, for its apparent lack of biological activity when *Asterias forbesi* toxin is injected into the red sea star, *Henricia sanguinolenta.*

Summary. A thermal toxin (or toxins) has been demonstrated in the coelomic fluid of scalded starfish, *Asterias forbesi,* which upon injection into normal starfish proved to be fatal and capable of inducing autotomy. Evidence suggests that this heat stable, dialyzable toxin was derived from epithelial cells lining the coelomic cavity. This material was toxic in several invertebrate species. Its chemical nature and mechanism of action are unknown.

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