The chromatophore system of the fiddler crab, *Uca*, has been the subject of several investigations. This crab blanches following the removal of its eyestalks. Extracts of the eyestalks (*3*) and central nervous organs (*4*) of *Uca* have darkening potency only. Several groups of investigators (*5*, *6*) have postulated a *Uca*-lightening hormone. The results of their experiments could be explained more simply by assuming that body lightening was due to a body-lightening hormone rather than to removal of the darkening hormone from the circulation. The following assumptions have also been made: (i) the darkening factor antagonizes the lightening factor so completely in a mixture of the two factors that only the darkening factor is able to express itself; (ii) when the crabs are dark there is a predominance of darkening hormone in the blood; (iii) when the animal is light there is an abundance of a lightening hormone in the blood. Transfusion of blood from a light crab to a dark crab has not confirmed the last assumption.

The experiment described here (*7*) was designed to demonstrate by perfusion the existence of body-lightening and body-darkening factors in the blood of the fiddler crab, *Uca pugilator*. The specimens were collected at Ocean Springs, Miss. *Uca* from the stocks in the laboratory were separated into two groups. The pigment in the melanophores of one group was maximally concentrated and in the second group the pigment was maximally dispersed. A crab whose melanin was maximally dispersed was induced to autotomize three walking legs. The most distal segment of each leg was then transected to facilitate perfusion. Each leg was then placed in sea water in separate Syracuse watch glasses. The first leg was perfused with 0.05 ml of blood taken from a *Uca* whose melanin was maximally concentrated. The second leg was perfused with 0.05 ml of blood from a *Uca* whose melanin was maximally dispersed. The third leg was perfused with 0.05 ml of sea water.

The chromatophore scale of Hogben and Slome (*8*) was used to stage the chromatophores. Stage 1 represents maximal pigment concentration, stage 5 maximal dispersion, and stages 2, 3, and 4 intermediate states of pigment dispersion. The stage of the chromatophores on the isolated legs was determined at 15, 30, and 60 minutes following the perfusion.

This experiment was performed 15 times. The averages for all the experiments are presented in Fig. 1. The results produced a family of curves. The pigment in the chromatophores on the legs of *Uca* gradually concentrates following isolation, as had been demonstrated previously (*6*). The pigment in the legs perfused with sea water concentrated slowly (curve *B*). Perfusion with blood from dark *Uca* slowed the rate of lightening as compared with the control legs (curve *A*). Perfusion with blood from a maximally light *Uca* caused a more rapid rate of concentration of the pigment in the isolated chromatophores than was observed in the controls (curve *C*).

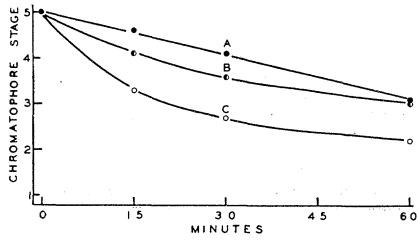

Fig. 1. State of dispersion of the pigment in the melanophores of isolated legs of the fiddler crab, *Uca pugilator*. (*A*) Legs perfused with blood from a maximally dark crab; (*B*) control, legs perfused with sea water; (*C*) legs perfused with blood from a maximally light crab.

These results could not have been obtained unless a lightening factor had been present in the blood of the pale *Uca* and a darkening factor had been present in the blood of the dark *Uca*.

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Detection of Complement-Fixing Antibodies for Carré's Virus

Carré's virus (canine distemper virus) produces the most common disease syndrome of the domestic dog, and it has also been suggested that it is capable of producing disease in the human being (*1*, *2*). Adams (*2*) has discussed the virus in relation to a specific respiratory disease process of man. Serologic techniques have been used for the detection of antibodies against the virus for many years. Early descriptions of complement-fixation and neutralization tests were given by Laidlaw and Dunkin (*3*), Pyle and Brown (*4*), and others (*5*). Little improvement was made in the basic techniques described by these early authors until Haig (*6*) discovered that Carré's virus could be adapted to the chorioallantoic membrane of the developing chick embryo. Cabasso and Cox (*7*) developed a serum neutralization test using this method.

Serum neutralization tests have the disadvantages of requiring large numbers of eggs or animals and, for the average laboratory, limiting the number of tests that can be done according to space and equipment. However, the increased interest in Carré's virus creates a need for a standard serologic test in order to compare and evaluate the results obtained in various laboratories. This report describes an antigen that can be used in a complement-fixation procedure that can be performed in most laboratories with minimal serologic equipment. It is interesting to note that since the completion of this work, Morris, Aulisio, and McCown (*8*) have described similar experiments that corroborate, in part, our results.

Seven-day-old embryonated chick eggs were used in preparation of the antigen. They were inoculated on the CA membrane with 0.1 ml of a 20-percent suspension of infected membranes. This material was prepared by removing membranes that showed a diffuse area of infection from eggs that had been inoculated 7 days previously. The membranes were ground in a blendor with enough saline to make a 20-percent suspension by weight. After 7 days' incubation at 37°C, the shells were removed, and membranes that showed multiple lesions were used for antigen production. Membranes were pooled, quick frozen, and stored at −20°C for at least 48 hours. After thawing, a 40-percent membrane suspension in buffered (*p*H 7.2) saline was made in a Waring-type blendor. Heavy particles were removed by centrifugation, and the supernatant fluids were stored in sealed ampoules. Normal membranes were similarly processed as control antigens.

Antiserums used in these trials were either stored at −20°C immediately after removal from the clot or first passed through a Seitz filter. Positive control serums were prepared by hyper-immunization of dogs with attenuated virus in ferret spleen preparations. The techniques of the tests were essentially the standard procedures described by Kolmer and Boerner (*9*). Overnight incubation at 5°C was employed in all cases.

Repeated titrations demonstrated that nonspecific reactions do not usually occur above a 1/8 dilution of serum. A 1/8 or 1/16 dilution appears to be satisfactory for single-tube screening-type tests. Positive reactions below this dilution are of doubtful significance. All positive serums should be titrated to determine endpoints.

Preliminary studies with the antigen in

the examination of human serum have indicated the presence of complement-fixing antibodies of significant level in some cases. In a group of 20 samples of normal individuals taken at random, 35 percent showed the presence of antibodies. In a group of 35 samples from patients with nonbacterial respiratory disease, the reaction level was 3 percent. In similar examination of 63 canine samples, where the reaction rate would be expected to be higher, a positive percentage of 45 was obtained.

There is no doubt that serologic methods for identification of Carré's virus antibodies require further improvement. An antigen giving specific reactions in greater serum dilution would be very advantageous. Perhaps more highly purified preparations would be more efficient. Adaptation of the virus to tissue culture may help solve some of these problems. With the realization of probable public health significance of the virus, the need for further immunologic and serologic studies is reemphasized.

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Observations at an Ancient Smelting Site in Negev

Prospecting for copper and other metals in Timnah area, Negev, is leading us to a closer study of some ancient mining and smelting sites. A reconstruction of ancient prospecting, mining, and smelting practices may prove to be of use in our current exploration. The present advance note (*1*) deals with some of our findings at a smelting acropolis (*2*) in the central part of Wadi Timnah (Meneiyeh Um Adak). A more detailed account is to be submitted elsewhere for publication (*3*).

Table 1. Chemical analysis of ores found in the Timnah acropolis (percentage of original sample). ND, not detected; P, Ti, HCl-soluble S, SO_4, and water-soluble Cl were not detected in any of the ores.

No.	CuO	MnO	Fe_2O_3	Al_2O_3	CaO	MgO	SiO_2	CO_2	Cl	Loss at 1100°C
348/55	19.3	0.09	23.9	1.3	1.4	0.4	43.2	3.9	0.8	12.5
349/55	23.4	ND	2.0	1.1	0.6	0.1	62.2	5.8	0.3	10.8
350/55	20.1	ND	3.3	0.9	7.8	0.2	52.1	10.6	0.3	16.0
351/55	24.2	ND	0.8	1.0	0.8	0.3	61.4	5.8	0.7	11.7
352/55	29.3	ND	0.6	1.1	0.7	0.3	54.8	7.8	0.1	14.9

Table 2. Some chemical data on concretionary copper ores containing chalcocite (percentage of original sample).

No.	Cu	S	SiO_2	CO_2	Cl	Loss at 1000°C
1416/55	38.5	2.4	35.4	8.9	1.95	18.4
1417/55	62.8	10.8	6.3	6.2	4.77	23.7
1029/55	58.7	6.9				
78/52	67.3	14.1		8.3		

The composition of the ancient ores and slags here reported poses more problems than it solves. Our success in following the ancient trails to the apparent sources of the ores found in the acropolis is in need of further evaluation. The quality of ancient slags and their remarkably high melting ranges (*4*) need to be reconciled with current views of ancient metallurgy. Finally, the out-of-place materials, other than artifacts, found in the acropolis cannot be accounted for in the present state of our knowledge. However, a preliminary report is not devoid of interest, in view of the cultural-historical importance of the area and the insufficiency of contemporary archeological knowledge.

Notwithstanding the opinion of such scholars as Glueck (*2*, pp. 77–79), mining in Timnah could not have been arduous at any time. Outcrops and tunnels utilized by our predecessors in mining required more skill than manpower in their discovery and development (*5*). Smelting of the ancient copper ores required far more skill than brawn, judging by the quality of the slags and of the finished products. Fuels and flux materials were procurable apparently within a short distance of the smelting site. The climate was pleasant in winter and easily tolerable in summer, even as it is now. All the operations for the production of copper in the ancient times could be accomplished by a handful of laborers, a few skilled technicians, and the military guard—essential then as it is today.

The acropolis containing ash and slag heaps is situated on a flat top of an isolated hill of white sandstone in the central part of the wadi. The hill is a rectangle, about 1000 by 400 feet, with vertical slopes about 100 feet high above the surface of the wadi. The hill can be ascended by one of the two paths cut or worn in the rock or by a talus slope from the south, by the side of a sand-filled cave now concealed by boulders and poorly accessible. The acropolis was visited by us five times in 1954–55. Collections of suitable materials were made for subsequent studies, and the largest slag heap was excavated in two places, to the sandstone floor of the hilltop.

Three types of materials were especially interesting in the earlier exploration: (i) fragments of copper ore on the surface of the ground and in the heaps, all of a uniform size but of five different kinds, morphologically; (ii) fragments of slag of two different kinds but of a rather small and uniform size, in contrast with other slag heaps in the area; and (iii) the out-of-place materials (*6*), notably fragments and masses of red sandstone and conglomerate containing small amounts of gold, as well as some other kinds of materials, including large crystals of calcite the sources of which are still undiscovered.

Fragments of the copper ores found in the acropolis were grouped into five categories, on the basis of their appearance and of the associated rock. The chemical analysis (*7*) of these ores is given in Table 1.

Outcrops of copper ore resembling the five kinds found in the acropolis were located by us within a short distance of the site, after a detailed search, involving utilization of ancient trails (along which sparse fragments of ores, calcite, and other materials were scattered), the normal dispersion trains, and other prospecting leads. Significantly, no chalcocite copper was found in the acropolis and it is possible that none was taken by the ancients of the acropolis from the outcrops or the wadis, despite its high copper content (Table 2) and its fairly common presence in some alluviums.