In the dog, both Orinase and its sodium salt were studied. They were equally effective in maintaining a lowered blood sugar, but the water-soluble salt decreased the blood sugar more rapidly. A single dose of 25 mg/kg of the sodium salt decreased and maintained the blood sugar of a normal starving dog at a level 25 to 30 percent below controls for at least 24 to 32 hours. When 100 mg/kg was given, a decrease of about 40 percent was observed for at least 32 hours (Fig. 2). Although 600 mg/kg produced a blood sugar depression no greater than 100 mg/kg, this dose caused death within 20 hours.

Rabbits that were given 400 mg/kg of the sodium salt of Orinase responded with maximum blood sugar depressions similar to those obtained with 100 mg/kg in dogs. The rate of recovery from hypoglycemia in these rabbits, however, was comparable to that in dogs that were given only 5 to 15 mg/kg. The lethal dose for rabbits was approximately 3500 mg/kg and, as in dogs, death was not the result of hypoglycemia.

Determinations of the plasma level of the drug in dogs that were given oral doses at levels of 5, 15, 25, 100 and 600 mg/kg indicated that at the peak plasma levels, about 10 percent of the dose can be found in the plasma (Fig. 2). The times required to clear the plasma of the drug were about 24, 48, and 72 hours for doses of 5, 25, and 100 mg/kg, respectively.

Chronic toxicity studies in several species are now in progress (*12*). Weanling rats of both sexes have been fed the drug for 8 weeks at approximately 100, 200, and 400 mg/kg, and no significant weight changes were seen when compared with controls. At 4 weeks, no change in the hemograms was apparent, but a moderate enlargement of the thyroid gland was observed in all rats that were given the higher doses.

The mechanism of action of Orinase has not been resolved at the present time, but it does affect the mechanisms involved in the deposition of liver glycogen in the fasting rat. The effect of Orinase on pancreatic function and on the peripheral action of endogenous insulin and glucagon is under study.

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23 February 1956

Insulin-Sparing Sulfonamides

An orally administered sulfonamide has been found to lower the blood sugar in normal individuals and in patients with "mild" diabetes (*1*). Toxic effects have been minimal or absent.

The present report (*2*) summarizes some observations of clinical interest in ten patients who have received one or both of two such sulfonamides (*3*). Six were "severe" diabetics, and four were "mild" diabetics. Three of the former and one of the latter have been studied under precisely controlled conditions on the metabolic ward.

Three of the five "severe" diabetics had very significant *decrease* in insulin requirement and/or lowering of blood or urine sugar on a constant dose of insulin. Two grams or more of sulfonamide per day were required to produce this effect. One "severe" diabetic on chemically constant intake had a significant *increase* in glycosuria during sulfonamide administration. The fifth "severe" diabetic had essentially no demonstrable effect.

Three middle-aged obese diabetics had more than 50-percent reduction in insulin requirement with sulfonamide administration of less than 2 g daily. One mild diabetic, maintained on chemically constant intake (without insulin), had a 50-percent reduction in blood sugar level during the day on which she received a single dose of 3 g of sulfonamide. A very diabetic glucose-tolerance curve reverted to normal following a single dose of 6 g of sulfonamide in one "preclinical" diabetic.

Twenty-four-hour iodine-131 uptake by the thyroid was diminished to less than 5-percent during intensive sulfonamide administration in three severe diabetics. With reduction in dosage, the uptake returned to a normal level.

In the three "severe" diabetics who responded favorably, a reciprocal relationship between blood free sulfonamide and blood sugar levels was noted.

In two "severe" juvenile diabetics, one of whom had profoundly favorable modification of the diabetic state, and the other of whom had a significant increase in glycosuria during sulfonamide administration, all of the administered sulfonamide could be accounted for in the urine. In the former, the greater portion of urinary sulfonamide was conjugated; in the latter, the greater portion was free.

Decrease in circulating granulocytes was noted during the administration of very large dosages of sulfonamides. Marrow findings were interpreted as showing maturation arrest (William Chew). The blood count returned to normal when medication was decreased or stopped.

The foregoing observations confirm the results recently reported by German investigators in patients with "benign" diabetes. In addition, the sulfonamides favorably modify the diabetic state in some severe diabetics. Significant differences in free and conjugated sulfonamide excretion, respectively, have been noted in severe diabetics who do and who do not respond favorably to sulfonamide. This suggests that differential metabolism of administered sulfonamide may be partly or completely responsible for the type of therapeutic response.

Large dosage may result in toxic manifestations. Reduction of dosage, thus far, has been associated with disappearance of such manifestations.

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2. We wish to acknowledge the technical assistance of Marjorie Coelho, George Fukayama, Florence Olson, and Evalyn Jones.
3. The two sulfonamides that have been used are BZ-55 (N_1-sulfanilyl-N_2-*n*-butyl-carbamide) and Orinase (a compound identical with BZ-55 except for the substitution of a methyl for the amino group on the benzene ring). Orinase was administered in doses ranging from 1 to 6 g/day. BZ-55 was administered in doses ranging from 1 to 16 g/day. Grateful acknowledgment is made to C. J. O'Donovan of the Upjohn Company for supplies of both preparations and to W. R. Kirtley of the Lilly Research Laboratories for supplies of BZ-55.

7 March 1956

Black Pigment Concentrating Factor in the Fiddler Crab

The production of both a body-lightening and body-darkening blood-borne factor by a crab has not been demonstrated although such antagonistic hormones are known in other crustaceans (*1*). All crabs that have been examined with the exception of three species of the genus *Sesarma* blanch following eyestalk removal (*2*).

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*).

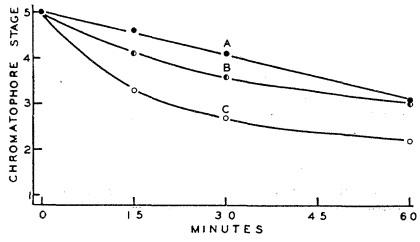

Fig. 1. State of dispersion of the pigment in the melanophores of isolated legs of the fiddler crab, *Uca pugilator*. (*A*) Legs perfused with blood from a maximally dark crab; (*B*) control, legs perfused with sea water; (*C*) legs perfused with blood from a maximally light crab.

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