from the swivel. A collar of tape which should not be applied tightly is used to secure the polyethylene catheter and its connection with the intravenous tubing.

The dog is placed in a loose-fitting harness which is connected to the inner cylinder of the swivel by a bicycle chain sufficiently long to allow the animal to lie down but which is shorter than the tubing between the dog and the swivel. Two points on the chain are connected by a piece of rubber tubing (B. Fig. 2) so that the excess chain and tubing are withdrawn from the cage when the dog is standing.

For protection, the intravenous tubing between the swivel and the dog is encased in heavy rubber tubing which is wired at multiple points to the bicycle chain except at the distal end where the excess tubing is allowed to accumulate. Slack in the tubing at this point eliminates the possibility of pull upon the connection between the intravenous tubing and the polyethylene catheter. When the dog lurches, his motion is thus stopped by the chain and no stress is placed upon this union.

Discussion. The swivel has been utilized in studies performed upon 10 dogs. These experiments ranged from 15 to 35 days in duration and were performed without mechanical difficulty. Only one experiment was terminated because the dog chewed the rubber tubing. The careful selection of gentle, quiet dogs will eliminate virtually all the difficulties one may expect with this mechanism. Two solutions were administered simultaneously and, in most experiments, rate of administration for each was controlled individually by glass stopcocks placed in the system between the fluids and swivel. Although this method was satisfactory, it required supervision because of fluctuations in rate of flow resulting from changes in the animal's position. More recent experience has demonstrated that rates of administration can be controlled with greater facility by the use of Sigmamotor infusion pumps.†

The effectiveness of the swivel was singularly dependent upon the bicycle chain. Its flexibility in one plane allowed the dog to stand or lie while its rigidity in all other directions provided an effective handle which rotated the swivel as the dog walked in the cage. Thus the bicycle chain was the link between the swivel and the dog which permitted ambulation without destruction of the intravenous tubing.

Summary. A method for continuous intravenous infusion of dogs confined to metabolic cages has been described. The method was free of mechanical problems and was entirely satisfactory in our experience. It can be recommended for any experiment requiring continuous intravenous infusion of dogs for indefinite periods of time.

† Sigmamotor, Inc., Middleport, N. Y.

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Role of Cations in Conglutination and in Formation of Properdin Zymposan Complex from Bovine Serum. (23432)

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Bovine serum is an inexpensive and convenient source of properdin(1). However, attempts to study the reaction of bovine properdin with zymosan were complicated by the conglutinating activity of bovine serum(1,2), which causes the clumping reaction known as conglutination. The studies reported here describe the methods developed to prevent conglutination.

Materials and methods. Sheep cells were preserved in Alsever's solution(3) containing penicillin and trisodium ethylenediamine tetraacetic acid(4). Standardized suspensions of sensitized sheep erythrocytes (EA), were

prepared as described by Osler *et al.*(5). Veronal buffer(6) containing 0.00015 *M* Ca^{++} and 0.0005 *M* Mg^{++} was used for dilutions and washing except where noted. All salts of E.D.T.A. were prepared from reagent $Na_2H_2E.D.T.A.$ and adjusted to pH 7.4-7.6 with NaOH. Bovine serum was stored at −70°C in small vials.

Results. Bovine serum was incubated at 37°C for one hour with zymosan,* 5 mg per ml of serum. 0.5 ml aliquots of the absorbed serum, and a control serum incubated without zymosan, were tested for any residual conglutinating activity by addition to 0.5 ml of EA and 1 ml of buffer and incubation at 37°C for 15 minutes. The absorption with zymosan removed the conglutinating activity showed by the control serum.

The role of Ca^{++} in conglutination was studied by adding $MgNa_2E.D.T.A.$ to a mixture of EA and bovine serum prior to incubation. The Ca^{++} was bound according to equation (1).

$$MgNa_2E.D.T.A. + Ca^{++} \rightarrow CaNa_2E.D.T.A. + Mg^{++} \quad (1)$$

$SrNa_2E.D.T.A.$ and $BaNa_2E.D.T.A.$, which chelate Ca^{++} in the same way(7) were tested along with a control tube containing $CaNa_2$ E.D.T.A. It was found that Ca^{++} is necessary for conglutination of EA. Mg^{++} and Ba^{++} are entirely ineffective while Sr^{++} appears to substitute to a limited extent for Ca^{++}. Similar results were obtained with zymosan.

The reversibility of conglutination may be demonstrated by adding $MgNa_2E.D.T.A.$ or $BaNa_2E.D.T.A.$ to conglutinated EA and incubating at 37°C for a few minutes. The clump disperses and no conglutination can be observed microscopically. After washing these dispersed cells several times with veronal buffer containing no divalent cations, resuspension in veronal containing Ca^{++} restores conglutination.

The reversibility of the reaction suggested that the components of the conglutinating system, except for Ca^{++}, remain bound to the cell during the dissociation phase; or that the components of the system are not bound, but react with, and alter, red cell components so that addition of Ca^{++} causes conglutination. None of the $MgNa_2E.D.T.A.$ washings of conglutinated cells showed any conglutinating activity with EA when Ca^{++} was added.

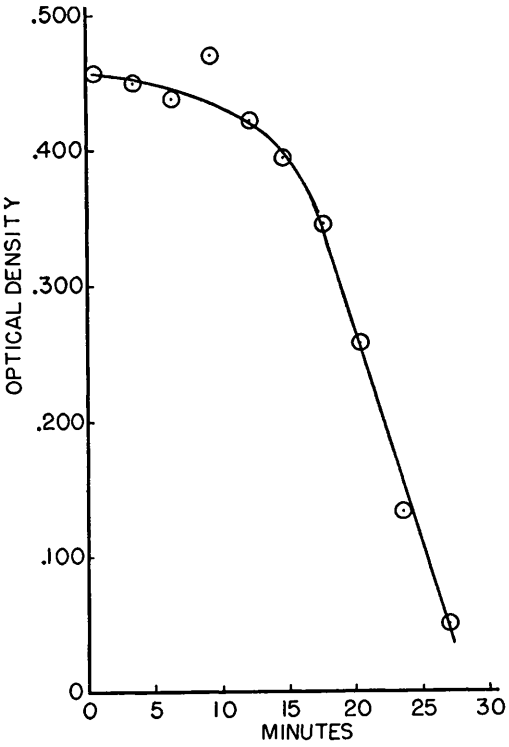

FIG. 1. Inactivation of C'3 by washed bovine PZ formed at 15°C for varying lengths of time. The optical density at 541 mμ measures liberated hemoglobin, and is proportional to C'3 concentration(8).

Since properdin requires Mg^{++}(1) and not Ca^{++} for its reaction with zymosan, the addition of $MgNa_2E.D.T.A.$ to bovine serum prevents conglutination of the zymosan, while permitting the zymosan to react with properdin. A kinetic study of the formation of properdin zymosan complex, in the presence of $MgNa_2E.D.T.A.$ at 15°C is shown in Fig. 1. The properdin zymosan complexes, obtained at various times, were incubated with C'3 (third component of complement) for one hour at 37°C. The residual C'3 was determined as described previously(8).

After absorption of properdin from serum containing $MgNa_2E.D.T.A.$, addition of fresh zymosan and Ca^{++} results in conglutination of the zymosan. Attempts to prepare active con-

* Purchased from Standard Brands, New York City.

glutinin by dissociation of conglutinated zymosan are in progress.

Summary. The conglutination of sensitized sheep erythrocytes or zymosan requires Ca^{++}. Mg^{++} and Ba^{++} are inactive but Sr^{++} appears to substitute to a limited extent for Ca^{++}. Zymosan inhibits conglutination of sensitized sheep cells by bovine serum. Bovine properdin and zymosan react readily in the absence of Ca^{++}, but in the presence of Mg^{++}, without any interference due to conglutination.

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A Method of Securing Bacteriologically Sterile Snails (*Australorbis glabratus*).* (23433)

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Recent progress in the axenic cultivation of multicellular organisms suggested that snails might also be cultivated in a bacteriologically-sterile environment. Accordingly, investigations were begun in 1954 to determine whether the eggs of *Australorbis glabratus,* the Western Hemisphere vector of schistosomiasis, could be induced to hatch under sterile conditions after being subjected to surface disinfection.

Materials and methods. 1. *Maintenance and handling of snails and snail eggs.* Eggs were derived from a Puerto Rican strain of *A. glabratus* maintained in our laboratory. The methods employed in rearing these snails have been described(1,2). Since large numbers of eggs were required, several 3-gallon aquaria were set aside for breeding purposes. Each aquarium contained 10 liters of water, a supply of water cress, and 25 snails which were replaced as they died or as fecundity diminished. Water cress was replenished at 5-7 day intervals at which time all egg-masses present on the vegetation were recovered by 'peeling' off the egg-masses using the edge of a watchmaker's forceps. The masses were then kept in a bowl of continuously aerated aquarium water at 76-78°F. Since snail embryos develop readily under these conditions, a supply was always available for experimental use (Fig. 1). Egg-masses containing immature embryos could not be employed since they commonly failed to hatch once disinfected. Consequently, when eggs were required, the stock was examined with a low-power microscope and only 'fully mature' masses selected. Such masses contained embryos which might be expected to hatch within 1-2 days; in fact, a crude guide to a 'mature' egg-mass was provided if one or more snails had already hatched at the time of examination (Fig. 2). The mature unhatched snail nearly fills its egg capsule, moves actively within its confines, and has a fully-formed shell which appears slightly yellow by indirect light. 2. *Composition and preparation of handling solution (HS).* The desirability of using a sterile solution of known composition in which disinfected snails would hatch was apparent. Such a solution was devised empirically by modifying 2 others which had been used, re-

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