body as used in this paper does not necessarily imply that each hemagglutin is physically and chemically separable into different components. It is entirely possible that the albumin, trypsin and Coombs' antibodies are the same as the saline variety but demonstrable only under special conditions. We have used the term in the latter sense until more specific data are available.

The dextran-active mouse hemagglutinin described by Gorer(1,2) in contrast to the one reported herein, was induced by tumor transplants. It was also quite labile on storage and of relatively low titre. Chattergee *et al.*(6) described a mouse, anti-sheep, and anti-rabbit hemagglutinin which was detected in tissue culture by the Coombs' test. Their data, however, were not entirely convincing. Owen(4) discussed a "blocking" and a Coombs' hemagglutinin in the serum of chickens immunized with human erythrocytes; his experimental data were not presented. An unsuccessful attempt to induce "incomplete" antibody in rabbits immunized with human erythrocytes was reported by Foster(7) together with qualitative differences in hemagglutinating versus hemolytic activity during the primary and secondary immune responses. With single injections of human erythrocytes into mice, we have not obtained antibodies with hemolytic properties. However, with sheep erythrocytes as antigen, a hemolysin is regularly induced.

Demonstration of a marked hemagglutin response to primary immunization which is not detected in saline will come as no surprise to those experienced with isoimmunization in pregnancy. One often receives human serums without saline antibodies which, on testing with trypsinized cells, with 20% albumin, or with an anti-globulin serum, are found to contain an Rh agglutinin at a titre of 1:1024 or greater(8). The presence of a similar situation in the mouse suggests that studies in which only the saline or hemolytic variety of antibody has been measured may require reexamination. Thus preliminary experiments with X-radiation of mice indicate that saline and trypsin hemagglutinin formation are inhibited to a greater degree than the albumin or the Coombs' response (Frisch and Davies, unpublished).

Summary. Injection of human, sheep and rabbit erythrocytes into Webster mice results in the appearance of "incomplete" varieties of hemagglutinins detectable in albumin, with trypsinized cells, and by means of a Coombs' reagent. In some animals given small doses of antigen, the saline agglutinin does not appear. At the same time, the "incomplete" ones may be present in high titre and escape notice unless tested for specifically.

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Received April 8 1959. P.S.E.B.M., 1959, v101.

Distribution of Ions in Intestinal Smooth Muscle.* (24912)

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The electrochemical properties of smooth muscle cells are so poorly understood that it is a question whether the relations between ions' distributions and transmembrane potentials is the same as, or merely similar to, the

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relation in other muscle and nerve cells. Bulbring's(1) and Holman's(2) results indicate that potentials of smooth muscle cells do differ from those of skeletal or cardiac in their magnitude and response to drugs and to changes in ionic concentrations in the external environment. Earlier measurements of total amounts of various ions in various smooth muscles were reviewed by Evans(3). When Manery(4) reviewed the subject of electrolyte metabolism little could be added other than that amounts of potassium in smooth muscle were smaller than in striated muscle while amounts of sodium and chloride were considerably greater. Whether larger amounts of sodium and chloride could be accounted for on the basis of a larger extracellular space remained a question. Daniel (5) has shown the difficulty of calculating intracellular ion concentrations on the basis of total amounts present in various smooth muscles and of assumptions which appear to be valid for striated muscle. In the present study an independent estimate of extracellular volume was made by using inulin as an impermeant molecule; total amounts of potassium, sodium, and chloride in pieces of intestinal muscle were determined; and calculations of intracellular ion concentrations were made.

Methods. Segments of ileum and jejunum were removed from young adult cats anesthetized with Nembutal. Cylinders of circular smooth muscle were prepared by stripping away the other intestinal layers(6). Circular muscle segments (0.1 to 0.3 g) to be analyzed for sodium and potassium were prepared quickly, blotted gently and weighed. After drying at 105°C for 24 hr the tissues were weighed again. Weight difference measured total water content. Tissues were ashed at 575°C, the ash dissolved in one drop of 0.1N HCl and diluted to either 10 or 25 ml depending on tissue weight. Sodium and potassium in samples were measured with Baird Associates flame photometer using a lithium internal standard. Standards contained both sodium and potassium in nearly the same ratio as found in muscle unknowns. Samples of blood were obtained by heart puncture. Plasma was obtained by centrifugation, dried, ashed, diluted and analyzed in the same way as muscle. Standards for plasma had appropriate sodium to potassium ratio. Samples for chloride analysis were prepared as above except that the tissues were dissolved in concentrated HNO_3 rather than ashed. The method of Schales and Schales(7) was used. Estimates of extracellular space were made from analyses of inulin content of tissues in equilibrium with 0.5% inulin in Tyrode's solution(8). Inulin used was obtained from Warner-Chilcott as 10% supersaturated solution in 0.5% sodium chloride. The method of Ross and Mokotoff(9) was used for inulin analyses.

Results. Average inulin space for 25 pairs of samples of circular intestinal muscle from 5 cats was 101 cc/kg wet weight. This tissue is apparently more compact than cat or rabbit myometrium, rabbit or guinea pig taenia coli (10) or frog stomach muscle(11). That enough time was allowed for the inulin space to be maximized is shown in Fig. 1. Since the chloride space is larger than the sodium space, intracellular concentration of chloride ions exceeds that of sodium ions. In addition, the sum of intracellular concentrations of potassium, sodium, and chloride ions in the intestinal muscle exceeds their sum in striated muscle(12). This difference is not much changed by using larger estimates of extracellular space in the calculations. Since the osmolality of internal and external solutions must be nearly equal, it must be concluded that there are fewer organic anions in intestional smooth muscle than in striated muscle.

Discussion. The data reported here differ from Daniel and Daniel's(10) results not in total amounts of potassium, sodium and chloride present in smooth muscle but rather in volume of extracellular space as approximated by an inulin space.

The chloride space cannot be used as a valid approximation of extracellular space because it exceeds the sodium space and thus calculations with it yield negative intracellular sodium ion concentration. Use of sodium space is not warranted since sodium is known to be contained in other muscle cells. Since the extracellular solution contains relatively more water and very little potassium, the calculated intracellular potassium concentrations

TABLE I. Distribution of Ions and Water in Cat Intestinal Circular Muscle.

	Intestinal muscle, meq/kg wet wt	Thigh muscle, meq/kg wet wt	Plasma, meq/l	Intestinal muscle, meq/kg cell H_2O
Potassium	77.0 ± 2.5 (27)	87.2 ± 1.8 (4)	3.7 ± .3 (8)	108 ± 6
Sodium	61.3 ± 1.5 (27)	35.2 ± 2.1 (4)	141 ± 3 (8)	65 ± 5
Chloride	66.6 ± 2.2 (13)		122 ± 2 (8)	77 ± 5
H_2O, g/kg	810 ± 2 (57)	804 ± 4 (4)	929 ± 2 (8)	
	cc/kg wet wt			
Inulin space	101 ± 17 (25)			
Chloride "	544			
Sodium "	432			

Numbers in parentheses indicate No. of measurements contributing mean values.

rise quickly to levels which indicate binding as larger values for extracellular space are assumed(10).

Conway(12) reviewed the methods and sources of error associated with estimation of extracellular space. He concluded that the inulin technic was as valid as any currently available. Calculations of intracellular concentrations based on an inulin space yield values for intestinal smooth muscle which are different than those for striated muscle.

Using intracellular concentration of potassium based on the inulin space a maximum diffusion potential of 84 mV may be calculated. Maximum values of the resting potential recorded in a parallel study to be reported elsewhere were less than 80 mV. Thus it would appear that in intestinal smooth muscle the potassium ion gradient is large enough to support the hypothesis that the resting membrane potential is essentially a diffusion potential dominated by the potassium gradient.

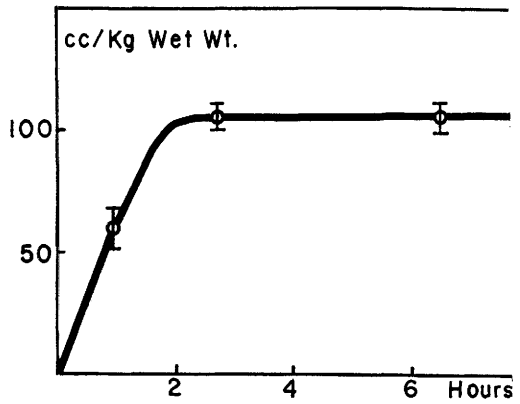

FIG. 1. The time course of inulin space shows the approach to equilibrium occurs after about 2 hr. Circles represent means of from 8 to 14 values and vertical lines represent stand. error of means.

Summary. Cat intestinal smooth muscle had an inulin space of 101 ± 17 cc/kg wet weight. Total water, potassium, sodium and chloride were determined and intracellular concentrations of potassium, sodium and chloride were calculated to be 108 ± 6, 65 ± 5, and 77 ± 5 meq/kg cell H_2O; respectively.

The author is indebted to Dr. C. L. Prosser for advice and encouragement.

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Received April 27, 1959. P.S.E.B.M., 1959, v101.