thalamic-pituitary-adrenal axis described by Urquhart *et al.*(3) and Yates *et al.*(4). It is also possible that both mechanisms occur simultaneously. However, the results of this study do confirm previous evidence that the liver plays an important role in regulation of plasma corticosteroid levels.

It is not likely that the observed decrease in hepatic metabolism of cortisol by these animals would precipitate an adrenal crisis during stress because there still remains a considerable capacity for steroid reduction in the salt deficient rats(5). Further studies under more severe conditions of sodium restriction and for longer periods might be valuable in assessing this effect in chronic sodium deficiency.

Summary. A decreased capacity of liver homogenate to metabolize cortisol in sodium deficient rats has been demonstrated. The rate of Ring A reduction was diminished by about 25% below that of control animals. The possibility that this effect may result in a decreased adrenal corticosteroid secretion *via* the negative-feedback mechanism by which plasma corticoid levels are stabilized is discussed.

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Received Sept. 9, 1960. P.S.E.B.M., 1960, v105.

Vi Antigens of the Enterobacteriaceae. IV. Purification and Properties of Vi Antigen of *S. paratyphi C*.* (26100)

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Isolation and purification of relatively undegraded Vi antigen by mild methods from *Salmonella typhosa, Escherichia coli* and *Paracolobactrum ballerup* has been reported by Baker *et al.*(1). Production of Vi antigen by some strains of *Salmonella paratyphi C* was reported by Rouchdi(2). However, neither the technic of purification mentioned above(1) nor that devised by Webster *et al.* (3,4) proved useable for purification of the Vi antigen of *S. paratyphi C*. In the present communication a method for the purification of the Vi antigen of this organism is outlined together with chemical and biological properties of this antigen.

* This investigation was carried out under the sponsorship of Commission on Immunization of Armed Forces Epidemiological Board and was supported by Office of the Surgeon General, Dept. of the Army.

Methods. The culture of *S. paratyphi C* (C-32) used was obtained from Dr. F. Kauffmann. The organisms were grown, as previously described(1), on a casamino acid medium containing only dialysable components. After about 18 hours growth the cultures were killed by addition of 1.5 to 2 volumes of acetone (technical grade), dehydrated by washing with acetone, and dried in air.

Nitrogen was determined by the usual micro-kjeldahl method(5), total acetyl as described by Kabat and Mayer(5), O acetyl by a modification of the method of Pippin, McCready and Owens(6), rhamnose by the method of Dische and Shettles(7), hexose by the method of Dische, Shettles and Osnos (8), and phosphorus by the method of Allen (9). Viscosities were determined by means of an Oswald pipette. The Ouchterlony technic was used for the serologic tests which

TABLE I. Analytical Properties of *S. paratyphi C* Vi Antigen.

Analysis	Lot E + F	Lot G
	%	
Nitrogen	5.85	5.8
Total acetyl	20.6	23.0
O acetyl	12.5	12.5
N " *	8.1	10.5
Rhamnose	.02	.02
Hexose	None	None
Phosphorus	"	"
Relative viscosity†	2.84	2 86

* N acetyl = Total acetyl − O acetyl.
† 0.2% solution in 0.15 M NaCl at 30°C.

were conducted as previously described(10).

Results. Attempts to purify the Vi antigen of *S. paratyphi C* by the previously described method(1) failed. Several preparations, when tested serologically by the Ouchterlony technic, showed the presence of several antigens other than Vi. Preliminary experiments indicated that the contaminating antigens could be eliminated early in the purification by treatment of the crude antigenic extracts with trichloracetic acid. To illustrate the method finally evolved, the following typical experiment is presented.

Seventy-five g of acetone dried bacilli were extracted twice with 750 ml of 0.15 M NaCl at room temperature. To the combined extracts cooled to 5°C, 0.25 N trichloracetic acid was added until no more precipitate formed. The precipitate was removed by centrifugation in the cold and discarded. The supernatant was neutralized to approximately pH 7 by addition of NaH CO_3. The antigens were then precipitated by addition of 2 volumes of ethanol. The precipitate was redissolved in 400 ml of 0.15 M NaCl. At this stage, the material contained at least 4 antigens when tested by the Ouchterlony technic. Further purification was carried out by the method used to prepare other Vi antigens (1). Briefly the method was as follows: A 2% solution of cetyltrimethylammonium bromide was added to the crude material until all of the Vi antigen was precipitated. The precipitate was washed several times with water, suspended in 0.15 M NaCl, and dissolved by addition of alcohol. The Vi antigen was recovered by addition of ethanol until no further precipitate formed (usually 3 to 4 volumes of ethanol were required). The precipitated Vi antigen was extracted at 37°C from the precipitate with 60% ethanol (60% by volume in 0.15 M NaCl). The extraction was repeated 4 to 5 times. The antigen was precipitated from the combined 60% ethanol extracts by cooling to 5°C. The precipitate was collected by centrifugation in the cold, and electrodialysed to remove the last traces of cetyltrimethylammonium bromide. The electodialysed solution was neutralized by addition of NaH CO_3, since the sodium salt was desired, dialyzed against distilled water, and dried from the frozen state. From 75 g of bacilli, 0.4 g of Vi antigen were obtained, a yield of about 0.5%.

Material in the supernatant from the cetyltrimethylammonium bromide precipitate was recovered by addition of 2 volumes of ethanol. This material was free of Vi antigen present in the original extract. This material has not been studied further.

The analytical values of 2 lots of Vi antigen, presented in Table I, were essentially the same as those previously reported for Vi antigens iseolated from *S. typhosa, E. coli* and *P. ballerup.*

The Vi antigen prepared from *S. paratyphi C* was non-toxic for rabbits in doses up to and including 10 mg. It did not prepare

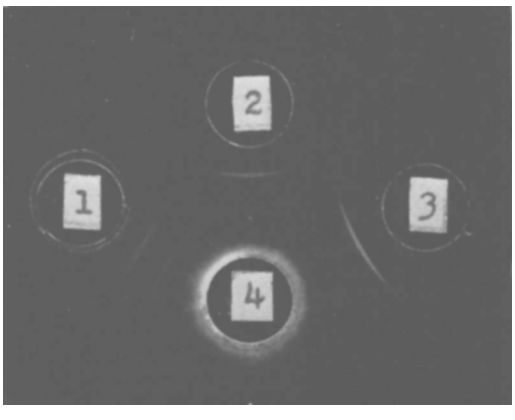

FIG. 1. Comparison of crude and purified *S. paratyphi C* Vi antigens with *S. typhosa* Vi antigen.
Reservoir 1 = Crude extract of *S. paratyhpi C.*
Reservoir 2 = Purified paratyphi C Vi antigen.
Reservoir 3 = Purified *S. typhosa* Vi antigen.
Reservoir 4 = *S. paratyphi C* antiserum.

rabbit skin for the Schwartzman reaction in doses of 250 μg. Pyrogenicity of the material was not tested. Serologic tests using the Ouchterlony technic are presented in Fig. 1.

Whereas there were several antigenic components in the crude material, only a single antigenic component was present in the purified antigen tested. Further, it was evident that the Vi antigen from *S. paratyphi C* was identical with that isolated from *S. typhosa*. Similar tests not presented here revealed an identity of the paratyphi C Vi antigen with those isolated from *E. coli* and *P. ballerup*.

Summary and conclusions. A method has been outlined for isolation and purification of the Vi antigen produced by *S. paratyphi C*. Consideration of both the chemical biological properties of this antigen indicated that it is very similar to, if not identical with, those produced by other *Enterobacteriaceae*.

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Received Sept. 15, 1960. P.S.E.B.M., 1960, v105.

Elimination of Circulating Serum Protein Antigens as a Sensitive Measure of Antibody.* (26101)

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The production of antibody following injection of soluble serum protein antigens into animals can be readily detected by the rapid elimination of the antigen from the circulation of the sensitized animal(1,2). This rapid elimination presumably results from the reaction of the circulating antigen with the newly synthesized antibody and uptake of the complex by phagocytic cells(1). Rate of elimination depends on amount of antigen injected, rate of antibody formation and perhaps quality of antibody produced(3). The accelerated elimination of serum protein antigens from the circulation also takes place following both injection of antigen in actively (4,5) and passively(4,6,7) immunized animals and injection of insoluble(8,9) and certain soluble(8) antigen-antibody complexes into normal animals. The purpose of the present investigation was to determine the minimum amount of passively transferred antibody which could be detected by the rapid elimination of I^{131} labeled antigen. The possibility of utilizing the rapid elimination of soluble antigens as a method to measure small amounts of antibody is discussed.

Methods and materials. I^{131} labeled antigens. Bovine serum albumin (BSA), Armour and Co., Lot No. S 68108, and bovine serum gamma globulin (BGG), Armour and Co., Lot No. C 904, were labeled with I^{131} (I*) as previously described(10).

* Publication No. 260 of Department of Pathology, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.

Supported by Atomic Energy Comm. contract AT (30-1)-1205.

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