that γ-resorcylic acid (2,6-dihydroxybenzoic acid) which has a stronger chelation effect than SAL, is more effective in the treatment of rheumatic disease. In the ascorbic acid depletion tests, reported in this study, γ-resorcylic acid is definitely less active than SAL. This agrees with a short statement in Reid's paper. Since the disassociation constant of organic acids is considered to reflect their chelation ability, the pK values, as reported in the literature, are listed in Table IV. It is apparent that the available figures do not indicate a relationship between the disassociation constant of these drugs and their effect on adrenal ascorbic acid.

Summary. 1. The effect of salicylic acid and similar compounds on the adrenal-pituitary system has been investigated. 2. Salicylic acid and a number of substituted salicylic acids cause a significant depletion of adrenal ascorbic acid. The only exceptions found so far are p-aminosalicylic and p-hydroxysalicylic acid. 3. The SAL blood levels parallel very closely the ascorbic acid depletion of the adrenal. 4. Hypophysectomy prevents the effect of salicylic acid on adrenal ascorbic acid. 5. Benzoic acid has an effect similar to that of salicylic acid, while several substituted benzoic acids, including p-aminobenzoic acid, are ineffective. 6. Twelve aliphatic acids of various types did not produce a change in adrenal ascorbic acid. 7. The experimental results are interpreted as indicating a specific direct or indirect action of salicylic acid on the anterior pituitary which results in the production or release of increased amounts of adrenocorticotropic hormone.

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Studies on Vi Antigen. I. Relative Vi Antigen Content of V Form Cultures. (19520)

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It has been demonstrated that vaccines prepared from rough Vi strains of *Salmonella typhosa*(1,2), and from Vi strains of *Paracolobactrum ballerup*(3,4) and *Escherichia coli*(4), none of which contain typhoid somatic components, are effective in the immunization of mice against infection with Vi + O strains of *S. typhosa*. Although 4 bacterial species are known to possess Vi antigen, information concerning their content of this component was lacking. The object of this investigation was to compare, by mouse protective potency tests, the Vi antigen content of these bacterial species to determine which provided the richest source of this antigen.

Cultures. The V form *S. typhosa* strains 58 and Ty2 were the challenge organisms used in the mouse protection tests. Strain 58 exhibited evidence of agglutinability by typhoid O antiserum, while Ty2 was quite resistant to O agglutination. When administered intraperitoneally in mucin, both strains exhibited maximum mouse virulence ($LD_{50} < 10$ organisms). When injected in saline the LD_{50} for strain 58 was approximately 220 million, while that for Ty2 was approximately 40 million. The other V form cultures used in this study were the Kauffmann strains *E. coli* 5396/38, *P. ballerup* 7851/39 and *S. paratyphi* C (East Africa). The properties of

these cultures have been described by Kauffmann(5). The only known antigenic component common to these bacterial species is the Vi antigen. Cultures were taken from the lyophilized state and maintained in the V form on nutrient agar (pH 7.6) by routine plating and colony selection. *E. coli* and *P. ballerup* cultures were incubated at 25°C, *S. paratyphi* C and *S. typhosa* strains at 37°C. Colonial morphology was examined with the aid of oblique illumination(6) and antigenic composition of each strain was checked periodically by tube agglutination tests with specific O and Vi antisera.

Mass culture production. Acetone killed and dried organisms were prepared as a convenient and stable source of Vi antigen since it had been reported(7) that V form *S. typhosa* prepared in this manner stimulated the production of Vi antibody in animals as readily as viable organisms. Prior to the mass culture of a given V form strain, the culture was plated, typical smooth dense colonies were selected and antigenic composition was tested until the strain was considered to be in the desired condition with respect to Vi antigen. Repeated colony selection was essential for the maintenance of the V forms of these strains. For large-scale production runs the organisms were cultured on veal infusion agar (pH 7.6) in Kolle flasks, from 18 hour seed agar cultures suspended in veal infusion broth, and incubated for 18 hours at the temperature appropriate for the species. Each step of the operation was checked by plating, examination of colonial morphology, microscopic examination of Gram stained preparations and agglutination tests with O and Vi antisera. When the control plates at the start and end of the production run revealed 95-99% V form colonies, this was taken as evidence that the harvest from the Kolle flasks likewise consisted of V form organisms in the same proportions. The growth was harvested in distilled water (10 ml per Kolle flask) and the organisms were precipitated from suspension with three volumes of acetone and collected by centrifugation. The organisms were then resuspended in fresh acetone and held at 37°C for 24 hours. Culture at this stage indicated that no viable organisms remained. The bacterial precipitate was collected on fritted glass filters. Final traces of acetone and moisture were removed by desiccation *in vacuo*. The total yield from 360 Kolle flasks was 60 to 90 g of dry organisms.

Vi antigen extracts. HCl extracts of the acetone dried organisms were obtained by the procedure employed by Luippold(8). Removal of the Vi antigen from the bacterial mass was facilitated by frequent shaking at room temperature for 24 hours. The resultant turbid suspension was cleared by centrifugation and then adjusted to pH 7.

Mouse protective potency tests. The assay procedure employed was an active-immunization mouse protection potency test based on the use of graded immunizing doses and a constant challenge dose(9). White Swiss mice, Bagg strain, weighing 14 to 16 g, and segregated as to sex, were assigned to jars in groups of 5. Assignment of animals to jars, assignment of jars to locations in the animal room, and the order of immunization and challenge injections, by groups, were all determined by randomization procedures. All immunizing injections were given intraperitoneally in a standard volume of 0.5 ml. Physiological saline was used as diluent. Four groups of 5 mice each, 2 groups of females and 2 groups of males, were employed for each dilution level tested. In individual assays, 3 or more levels of 4- or 5-fold progressive dilutions were employed. The mice were challenged 6 days after the immunization by the intraperitoneal route. Two types of challenge suspension were employed, 1000 *S. typhosa* 58 suspended in 5% gastric mucin, and 100 million *S. typhosa* Ty2 suspended in saline. The period of observation following challenge was 3 days.

Since the assay procedure was based on protection against typhoid challenge, and since the comparison of the Vi content of the various V form organisms with that of *S. typhosa* was the factor of prime interest, acetone dried strain Ty2 (Table I) and an HCl extract of strain 58 (Table II) were adopted as reference standards for comparison of the 3 sources of Vi antigen. The method of Knudsen and Curtis(10) which involves transformation of the survival data to angle values was employed to calculate the ED_{50} and relative potencies of these preparations.

TABLE I. Survival of Mice Immunized with Acetone Killed and Dried V Form Cultures and Challenged with *S. typhosa* Ty2.*

V form culture	Acetone dried organisms, immunizing dose (μg)						E.D.$_{50}$ (μg)	Relative potency (*S. typhosa*=1)
E. coli 5396/38	.02	14/20†	.004	9/19	.0008	4/20	.0094	6
P. ballerup 7851/39	.10	17/20	.02	11/20	.004	5/20	.0149	3.2
S. paratyphi C (East Africa)	2.5	8/20	.5	5/20	.1	1/20	4.340	.02
S. typhosa Ty2	.5	18/20	.1	15/20	.02	5/20	.0504	1

* Challenge dose, 140000000 organisms suspended in .5 ml of saline inj. intraper.
† Numerators denote No. of survivors at 72 hr; denominators, total mice tested.

TABLE II. Survival of Mice Immunized with HCl Extracts of V Form Cultures and Challenged with *S. typhosa* 58.*

V form culture	1% HCl extract, immunizing dose (μg) †						E.D.$_{50}$ (μg)	Relative potency (*S. typhosa*=1)
E. coli 5396/38	.120	19/20‡	.030	11/20	.0075	4/20	.022	12
P. ballerup 7851/39	.299	18/20	.075	14/20	.019	4/20	.049	5.5
S. paratyphi C (East Africa)	51.5	18/20	5.15	8/20	.515	2/20	6.100	.04
S. typhosa 58	1.65	17/20	.165	8/20	.0165	1/20	.257	1

* Challenge dose, 1000 organisms suspended in .5 ml of 5% mucin, inj. intraper.
† Extracts tested in the fluid state. Solids determined by drying extracts at 90°C to constant wt.
‡ Numerators denote number of survivors at 72 hr; denominators, total mice tested.

Results. The results of mouse protective potency tests on animals immunized with acetone killed and dried V form coli, ballerup, typhoid, paratyphi C and with HCl extracts of these organisms are detailed in Tables I and II. These tests were repeated on several occasions with similar results. In each experiment the four cultures were assayed simultaneously in order that their protective potencies might be compared directly.

The data given in Table I show that acetone dried *E. coli* was the best protective antigen. In terms of relative potency it possessed twice the activity of *P. ballerup* and six times that of *S. typhosa* Ty2. In contrast, *S. paratyphi* C showed only a trace of activity. The data in Table II indicate that the *E. coli* extract with a relative potency of 12 was the most active antigen, being approximately twice as potent as the *P. ballerup* extract and twelve times as active as the extract from *S. typhosa* 58. *S. paratyphi* C possessed minimal activity. The relative potencies of the four extracts corresponded reasonably well to the values obtained with the intact organisms which suggests that extraction of Vi antigen from these organisms was fairly uniform.

The protection against typhoid challenge afforded by these organisms and extracts was considered to be related to the quantity of Vi antigen contained in the immunizing dose. It was assumed that the observed differences were a reflection of quantitative differences in Vi antigen content of these strains. The possibility remains that there may be qualitative differences as well. However, no evidence to support the latter possibility was obtained in this study.

These findings in addition to providing confirmation of the effectiveness of *E. coli* 5396/38 organisms and extracts in protection of mice against typhoid challenge(4,8), show by direct comparison, in quantitative bioassays, the extent to which this strain of *E. coli* surpasses *S. typhosa* and the other V form species as a source of Vi antigen for the purposes of concentration and isolation.

Summary. The known V form non-typhoid organisms, *E. coli, P. ballerup* and *S. paratyphi* C in the form of acetone dried organisms or HCl extracts are all effective in the immunization of mice against typhoid challenge. Their relative potencies in this respect, compared with an arbitrarily fixed value of 1

for acetone dried *S. typhosa* Ty2 are as follows: *E. coli,* 6; *P. ballerup,* 3; *S. paratyphi* C, 0.02. It thus appears that *E. coli* 5396/38 is the richest source of Vi antigen among the known V form *enterobacteriaceae.*

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Failure to Demonstrate *in Vitro* Lysis of Sensitized Guinea Pig Leucocytes by Dog Hemoglobin Antigen.* (19521)

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Squier and Lee(1) have reported demonstration of a mean average reduction of 43% in the total number of leucocytes resulting from the addition of short ragweed antigen to heparinized whole blood from patients sensitive clinically and by skin test to the ragweed pollen. This reduction affected chiefly the polymorphonuclear leucocytes. It was postulated that the leucocyte reduction was due to an antigen-antibody reaction. It was further postulated that the antibody in this instance was a thermolabile reagin inactivated by heating to 56°C. Franklin and Lowell have failed to duplicate these results(2). This report describes an attempt to apply Squier and Lee's technic to a study of some phases of hypersensitivity and anaphylactic shock, using mature guinea pigs.†

Method. The hemoglobin suspension utilized as antigen in this experiment was prepared from citrated whole blood of dogs. The erythrocytes were separated by centrifugation and washed 3 times with normal saline, following which they were lysed with distilled water. Sufficient 18% NaCl was added to make a slightly hypertonic solution and again centrifuged to precipitate the stroma of the erythrocytes. The supernatant was drawn off and to this added about ⅓ its volume of "Amphogel". After standing for about 2 hours this latter mixture was filtered through coarse filter paper. The filtrate contained nearly pure hemoglobin and was utilized as antigen in the experiment. Twelve guinea pigs ranging in weight from 300 to 400 g were sensitized by 2 subcutaneous injections of 0.2 ml portions of the hemoglobin antigen at 48-hour intervals. Ten control guinea pigs ranging in weight from 300 to 350 g were similarly injected with 0.2 ml portions of normal saline. In the experimental group 5 females and 7 males were used. In the control group 5 females and 5 males were used. These animals were caged in groups of 5 and the sexes kept separated. None of the females were known to be pregnant. The diet was considered adequate and all of the animals were apparently healthy. In the control group blood was drawn by intracardiac puncture on the 13th or the 15th day after the second subcutaneous dose of saline. In the experimental group blood was similarly drawn on the 20th, 21st, or 32nd day after the second sensitizing dose of hemoglobin. The experi-

* This study was carried out under contract with the Division of Biology and Medicine, U.S.A.E.C.

† Animals obtained from Carworth Farms.