

sure at which the animal either died or collapsed. Using chicks, the pressure at which the animals fell backward was decided upon as the critical pressure since this pressure is very close to death and represents a definite end-point. The pressure at which the last extensor thrust of the hind legs occurred was chosen as the end-point in death of the mice.

Using these two criteria as end-points chicks singly and mice in pairs were decompressed in atmospheres (1) of air and (2) of oxygen. To make certain that the animal jar contained as nearly pure oxygen as possible for the latter experiment it was washed out with approximately 14 times its own volume with oxygen and then a constant stream of oxygen passed through it during the trial. Samples of gas in the chamber were also analyzed with a Haldane apparatus to make certain of oxygen saturation. Since the rate of decompression was kept the same in all experiments by an inlet valve it can be seen that the same rate of inflow of air or oxygen existed throughout all the trials. Therefore at any given barometric pressure the only difference in the animal jar was the pO_2 . All experiments were conducted at room temperature of $26^\circ C$. Each experiment was started at 740 mm Hg pressure and time was allowed for the animals to become adapted to the starting pressure. It is doubtful if this precaution was necessary

because the natural barometric pressure varied between 741 and 751 and therefore there was no significant pO_2 difference.

An examination of the data (Table I, B) shows a considerable species difference in the anoxic resistance of mice and chicks. Wright⁷ computes that 110 mm is the lowest barometric pressure at which a person breathing pure oxygen can remain conscious. He calculates that hyperventilation by washing out CO_2 from the blood could reduce alveolar pCO_2 from 40 to 23 mm. It has been stated⁸ that pO_2 of alveolar air at the time of death is probably about 10 mm Hg. The very rapid polypneic breathing of mice undoubtedly accounts partly for their ability to tolerate lower barometric pressures than the chicks. Hailman's³ data for rats decompressed in oxygen compare favorably with ours for mice, there being only about 5 mm difference at death.

Summary. Mice and chicks were decompressed in atmospheres of (1) air and (2) oxygen and the critical pressures determined below which the animals could not survive. A considerable species difference exists.

⁷ Wright, S., *Applied Physiology*, 1941, N. Y., Oxford Univ. Press.

⁸ Selladurai, S., and Wright, S., *Quart. J. Exp. Physiol.*, 1932, **22**, 233.

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Relation of Number of Injections to the Titer of Sperm Iso-agglutinins. in Mice.*

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It is well known that certain animals can form antibodies against their own spermato-

zoa¹ or against the spermatozoa of other individuals of the same species.² In the case of the species heretofore used (guinea pigs, rabbits, rats) relatively few injections have suf-

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New Jersey College for Women where some of the experiments were carried out.

¹ Metalnikoff, S., *Ann. Inst. Pasteur*, 1900, **14**, 577.

² Henle, W., *J. Immunol.*, 1938, **34**, 325.

TABLE I.
Titers of Sperm Agglutinins Produced by the Injection of Mouse Sperm Into Mice.

Total No. of inj.	Approx. No. of sperm per inject., $\times 1,000,000$	Injections	No. of $\varnothing$ mice inj.	Titers of sperm antisera*
(a) Intraper.				
4	30	every other day	1	<1:3
4	90		1	1:3
4	270		3	1:3 $\varnothing$ died $\varnothing$ died
5	30		1	<1:3
5	90		3	<1:3 <1:3 <1:12
7	30		1	1:3
7	90		1	1:48
12	{ 3 of 10 3 of 20 3 of 30 3 of 50	Wed., Thurs., Fri.	5	<1:24 <1:24 1:6 1:24 1:48
15	30		10	1:48 1:48 1:48 1:48 1:96 1:192 1:192 1:384 1:384 >1:1536
21	10		5	1:48 1:192 1:192 1:384 1:384
21†	{ 18 of 10 3 of 30		4	1:384 1:384 1:768 1:768
20-21	30		7	1:96 1:768 1:768 1:768 1:1536 1:1536 1:3072
21	90		1	1:48
(b) Subcut.				
15	35	Wed., Thurs., Fri.	5	<1:3 <1:3 <1:3 <1:3 <1:3

* Defined as the highest dilution of antiserum giving +++ agglutination.

† First 4 injections were intravenous.

ficed for the formation of antibodies. The experiments with mice here reported show that for this species repeated injections are necessary for the formation of anything other than very low titers, and that with the use of repeated injections high titers of sperm iso-agglutinins can be developed.

Methods. Sperm for injection were obtained by killing male mice, dissecting out the vasa deferentia and epididymides, mincing these in Locke's in a watch glass with fine scissors, straining through 2 fine wire strainers (75 and 112 wires to the inch respectively) into a filter flask, centrifuging to remove excess fluid and resuspending in the amount of Locke's desired for injection. The sperm yield as determined by counts with a hemocytometer ranged from about 18,000,000 to 50,000,000 per male, the average being about 30,000,000. Most injections were intraperitoneal, a few subcutaneous or intravenous. Injections were made every other day, or in courses of 3 consecutive days followed by 4 days' rest.

The males used to provide sperm were predominantly from the C57 black or BBC strains, the latter being a hybrid derivative of the C57 black strain, but some animals of

other stocks were used. The animals injected were in every case B alb C (MacDowell-Bagg albino) females. They were killed 7 to 14 days after the last injection.

Titer tests were run in 1 cc vials. Sperm were obtained by stripping vasa deferentia into Locke's, 0.6 to 0.8 cc of Locke's being used for each 2 vasa. After shaking, 0.1 cc of the resulting sperm suspension was added to 0.2 cc of each serum dilution. Readings were made after 15 to 30 minutes from a drop under the microscope. The degree of agglutination was classified as 0, +, ++, +++, or +++++, the latter indicating that all or nearly all of the active sperm were stuck, usually by the tail or middle-piece, to other sperm. The activity of the sperm was invariably unimpaired as compared with the controls so that the usual picture consisted of clusters or mats of actively beating but firmly enmeshed sperm.

Results. The results show that when 7 or fewer injections were given the sera were usually negative, though a few titers of 1:3 and one of 1:48 were obtained. Twelve injections over a period of 4 weeks gave titers of 1:48 or lower. However, 15 injections over

a period of 5 weeks gave titers of 1:48 to 1:384 (and one exceptional titer of >1:1536) and 21 injections over a period of 7 weeks gave titers of 1:48 to 1:3072, the majority being 1:192 to 1:768.

The optimum dose appears to be from 10,000,000 to 30,000,000 sperm per injection. Two of 3 animals injected with 270,000,000 sperm per injection died after the fourth injection. One animal given 21 injections of 90,000,000 sperm each over 7 weeks yielded serum with the low titer of 1:48.

Subcutaneous injections failed to produce agglutinins.

All controls were negative except that one control serum was found to have a titer of 1:96. Also one lot of pooled serum from 18 untreated males used in another experiment

contained sperm agglutinins. The reason for these rare agglutinins in sera of untreated animals is unknown.

Absorption tests with some of the sera produced in the above series of experiments have yielded evidence of the existence of an antigen or antigens in the sperm of C57 black mice not present in the sperm of B alb C mice. These tests will be described in detail elsewhere.

Summary. Four or 5 intraperitoneal injections on alternate days of mouse sperm into mice failed to produce more than a very low titer of sperm agglutinins. However, with 21 injections over a period of 7 weeks titers of 1:48 to 1:3072 were developed. Subcutaneous injections failed to lead to the formation of antibodies.

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A New *Salmonella* Type: *Salmonella mississippi*.*

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Among a lot of *Salmonella* cultures received from the Mississippi Department of Health was one isolated from a stool specimen of a normal food handler. This bacillus was identical with none of the previously described *Salmonella* types and to it was assigned the name *Salmonella mississippi*. It was a motile rod which in its morphological, cultural, and biochemical characters conformed to the usual pattern of paratyphoid bacilli. Glucose, arabinose, xylose, rhamnose, maltose, dulcitol, and sorbitol were promptly fermented with the production of acid and gas. Lactose, sucrose, salicin, adonitol, and inositol were not attacked. The bacillus fermented d-tartrate, mucate, and citrate but did not utilize l-tartrate. Large amounts of hydrogen sulphide were produced in peptone broth but indol was not formed.

When examined serologically the organism was agglutinated strongly by O serums derived from *S. worthington* (I, XIII, XXIII) and *S. poona* (XIII, XXII). It was agglutinated to a lesser degree by serums which contained agglutinins for antigen I. When tested with single factor serums for antigens XXII and XXIII, agglutination occurred in both. Absorption of *S. worthington* O serum with *S. mississippi* resulted in a complete removal of agglutinins, but the bacillus did not remove all agglutinins from *S. poona* O serum. When an O serum was prepared from *S. mississippi* it was found that neither *S. poona* nor *S. worthington* was able to exhaust it of agglutinins, although when used in combination the organisms left only a slight residual titer in the serum. It is obvious that the O antigens of *S. mississippi* are complex and that the O antigens of the previously described members of the *S. poona*-*S. worthington* group cannot be fully expressed by the symbols hitherto assigned to

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