oxidation of ethyl alcohol (about 4.5 mM per kilo per hour) is less than one-half that of the maximum rate of oxidation of sugar in the normal animal. The rate of acid production, assuming that all of the alcohol (4.5 mM) is converted into acetic acid, is relatively small and is well within the capacity of the animal to remove all of the acid by oxidation.

Summary. 1. A procedure is described for the determination of the free volatile acids of tissues. 2. Normal tissues of the dog, with the exception of brain, contain only traces, from 0 to 0.25 mM or cc *N*, of free volatile acidity per kg. This constitutes from 1 to 3% of the total volatile acids which can be obtained by hydrolysis with 2 *N* H_2SO_4. About 10%, from 1.5 to 2.0 mM, of all of the volatile acids of the brain are present either as free acid or in some form which is readily hydrolized. 3. The metabolism of ethyl alcohol in the dog does not result in an increase of the free volatile acids of the blood and tissues.

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Evidence of Local Protection Against Infection with Type I Pneumococcus.*

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The production of local protection or "local immunity" to microorganismal infection was first fully described by Wassermann and Citron[1] and interpreted by them as an *"Umstimmung"* or "retuning" of the local cells. This phenomenon has subsequently been observed but differently explained, especially by Besredka,[2] Gay,[3] and Opie.[4]

Bull and McKee[5] and recently Walsh and Cannon[6] demonstrated in rabbits a definite specific resistance of the upper respiratory tract to pneumococcal infection subsequent to specific local intranasal instilla-

* Aided by a grant from the David Trautman Schwartz Research Fund.

1 Wassermann, A., and Citron, J., *Z. f. Hyg. u. Infectionskr.*, 1905, **50**, 331.

2 Besredka, A., *Compt. Rend. Soc. de Biol.*, 1923, **88**, 1273.

3 Gay, F. P., *The Newer Knowledge of Bacteriology and Immunology*, Jordan, E. O., and Falk, J. S., University of Chicago Press, 1928, 881.

4 Opie, E. L., *J. Immunol.*, 1929, **17**, 329.

5 Bull, Carroll G., and McKee, C. M., *Am. J. Hyg.*, 1929, **9**, 490.

6 Walsh, T. E., and Cannon, P. R., *J. Immunol.*, 1936, **31**, 331.

tion. These observers point out that although the rabbit host seems protected in general, evidences of mouse-protective substances are not demonstrable. Gay and Rhodes,[7] on the other hand, found that only the derm was resistant to the streptococcus following experimental erysipelas whereas infection was produced by other routes of inoculation. Extensive literature in this field is afforded by Pacheco[8] and Gay.[9]

We employed both hairy and hairless white mice, and Type I pneumococcus A5-51R obtained through the courtesy of Dr. Kenneth L. Burdon and grown on blood-agar slants. When a saline suspension of the pneumococcus killed with 0.4% formalin was injected intraäbdominally on each of 5 days during a period of one week, the animals then survived for 5 to 6 days subsequent to an intraäbdominal injection of living pneumococci that was fatal to controls over night. In seeking a useful ratio of protecting factors to lethal dose it was found that .05 cc of saline suspension of a 24-hour growth, containing approximately one-half billion microorganisms per cc, killed mice within 24 to 48 hours when injected intraäbdominally, thus representing one minimal lethal dose. A similar dose injected subcutaneously killed in 36 to 72 hours. It was further ascertained that mice receiving 5 0.5 cc intraäbdominal injections during a week, of the formolized suspension containing approximately one billion per cc were protected practically 100% against an intraäbdominal injection of .05 cc of living cocci in suspension of about one-half billion per cc. With this standardization of protective procedure, the animals were grouped in various series representing similar routes of protective and lethal dosages and also divergences of these routes.

In Series 1, the routes of both injections, killed and living, were intraäbdominal; in Series 2, the protective injections were intraabdominal and the lethal doses were subcutaneous; in Series 3, both were subcutaneous; in Series 4, the protective injections were subcutaneous and the lethal were intraäbdominal.

Series 1: Ten mice received 5 intraäbdominal injections of the formolized suspension during one week, then .05 cc of the living suspension was administered intraäbdominally. All these animals survived.

Ten controls, 5 of which had been given 5 intraäbdominal injec-

[7] Gay, F. P., and Rhodes, B., *J. Infect. Dis.*, 1922, **31**, 101.

[8] Pacheco, G. A., *Arch. Path.*, 1932, **13**, 868.

[9] Gay, F. P., and Associates, *Agents of Disease and Host Resistance*. Charles Thomas, Baltimore, 1935, 448.

tions of formolized saline solution, received .05 cc of the living suspension and all died within 24 to 48 hours.

Series 2: Five mice received 5 intraäbdominal injections of formolized suspension during one week, then .05 cc of the living suspension was administered subcutaneously. Four of the animals died within 4 days and one in 10 days.

Series 3: Five mice received 5 subcutaneous injections of formolized suspension. On the following day, .05 cc of the living suspension was given subcutaneously. The protective injections were closely grouped and contiguous, and the lethal dosage was injected within this area. All 5 were protected while 4 controls all died within 48 hours.

Series 4: Five mice received 5 subcutaneous injections of formolized suspension during one week. The following day .05 cc of the living suspension was given intraäbdominally. Four mice died within 3 to 5 days and one survived for 12 days.

Walsh and Cannon[10] found in rabbits that at least 5 daily nasal applications of the antigenic preparation were necessary for protection against the pneumococcal infecting dose. Barach[11] obtained some degree of protection in mice 3 days following one intraäbdominal protective dose, and demonstrated that it was type-specific. Inasmuch as the peritoneal cavity permitted of better retention of the stimulating or possibly desensitizing factor, the following tests were made:

Two mice received .5 cc of killed suspension intraäbdominally and, after 24 hours, were given the infecting dose by the same route. Both animals survived.

Two mice received a similar protecting injection and the infecting dose at the same time. Both died in 48 hours.

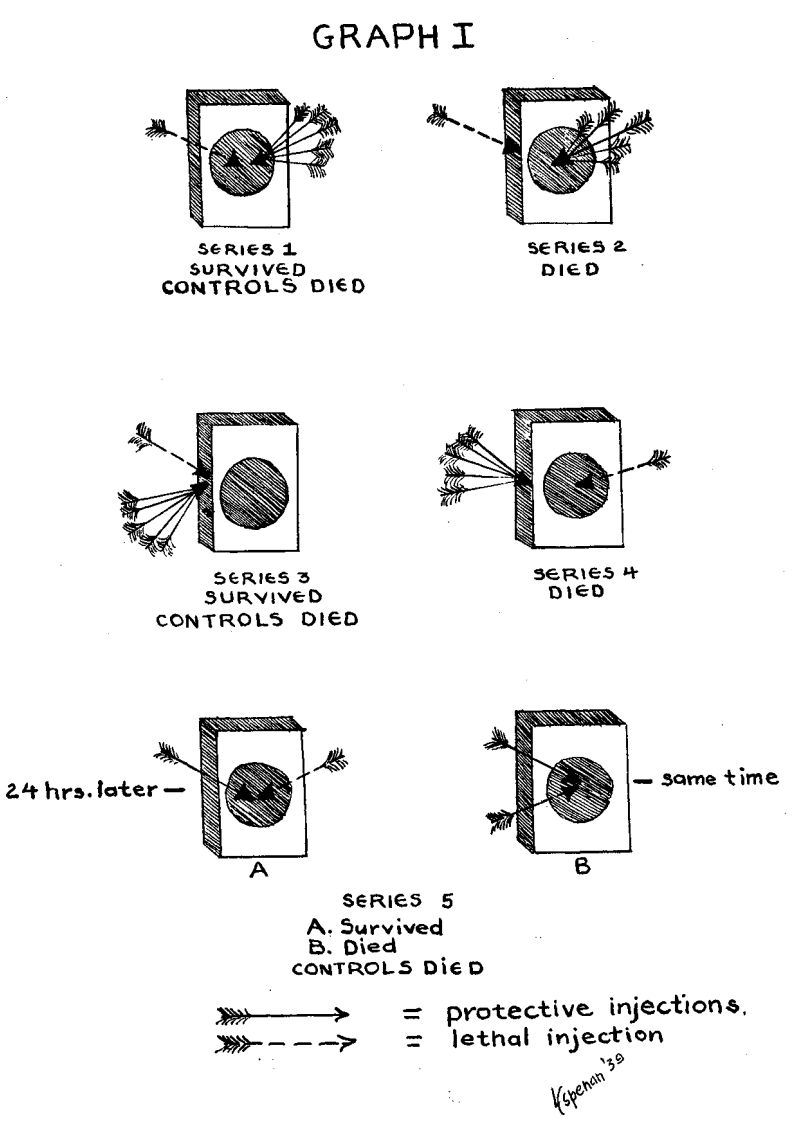
Two controls received the identical infective dose and both died in 48 hours.

These experiments have been confined to the subject of local protection as contrasted with systemic immunity. No tests of specificity have been carried out.

Duration of protection of the 10 surviving mice in Series 1. Groups of these were given another injection of living pneumococci after 1, 2, 4, and 6 weeks. At 6 weeks, the resistance had abated inasmuch as the animals died after from 4 to 6 days, whereas controls died within 48 hours. A few tests of the peritoneal fluid of such

[10] Walsh, T. E., and Cannon, P. R., *J. Immunol.*, 1936, **31**, 331.

[11] Barach, A. L., *J. Exp. Med.*, 1928, **48**, 83.



protected animals have failed to show capsular swelling or bactericidal properties.

From these results it is concluded that local protection against Type I pneumococcus is producible in the white mouse whereas infection and death occur from injections administered in untreated areas, thus indicating absence of, or at least deficient, systemic protection.

The duration of this protection extends from 4 to 6 weeks.

The protection of mice with one injection of the formolized suspension just 24 hours prior to the administration of an infecting

dose that kills controls within 48 hours, would appear to question seriously the formation of active immune factors, either humoral or cellular. It is suggested that the "shock-tissue" of the particular area is desensitized to the living pneumococcus by the primary injection of the formolized suspension.

It seems likely that with the recent interest awakened in this field that practical application will be attempted especially in the specific prophylaxis of those diseases gaining access through the upper respiratory tract, notably meningococcal meningitis and anterior poliomyelitis.

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A Simple Method for Accurate Injection of Small Volumes of Fluid.

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In order that the volume of dye, patent blue V, injected intradermally during studies on the linear rate of lymph flow in the superficial lymphatics of the skin of man by the McMaster[1] method, might be constant and accurate, an attachment was constructed to fit an ordinary Becton-Dickinson tuberculin syringe. The piece of apparatus proved to be simple, accurate, and very useful for these studies, and especially advantageous for colored solutions which preclude the reading of the scale on the cylinder of a syringe. Since it might prove useful to others whenever very small volumes of fluid are to be accurately measured and delivered with constancy into the tissues of the organism, the apparatus was considered worthy of report.

The attachment consists essentially of 3 parts (Fig. 1) which are mounted and used as follows: The cylinder of a tuberculin syringe is slipped into part *e*, which is fixed to the lip of the cylinder by screwing part *b* into *e*. Part *a* is then screwed on part *e*. The plunger is then inserted through the central openings in parts *a*, *b* and *e* and into the cylinder of the syringe until its head rests against *a*. The outer circumference of *a* was marked off in equal parts so that with the aid of a pointer *d*, fixed on *e* by screws, whole and fractions of a turn of *a* could be determined. The threading on *a* and *e* is such that one com-

[1] McMaster, P. D., *J. Exp. Med.*, 1937, **65**, 347.