The Critical-Orifice Liquid Impinger as a Sampler for Bacterial Aerosols[1]

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Studies in aerobiology are often conducted on a comparative basis; that is, the numbers of viable airborne organisms obtained under one set of circumstances are compared to those obtained under other conditions. For samplers employed in such studies, reproducibility and ease of handling often are considered more important than absolute efficiency because the final conclusions are derived from internal comparisons between various data collected using the same samplers throughout. In planning studies at Georgia Institute of Technology on the effect of chemical vapors on the viability of airborne bacteria, the critical-orifice liquid impinger was selected because of the demonstrated reproducibility and ease of handling (Rosebury 1947; Henderson 1952). Subsequent experience justified this choice, although it was found that the efficiency was significantly affected by the composition of the liquid used in the sampler. Further studies showed that this effect was responsible for potentially large errors when estimating particle size by the method of comparing aerial concentration to number settling per unit area per time unit. This experience indicated the need for consideration of other factors which might influence the operation of critical-orifice liquid impingers when used as samplers for bacterial aerosols.

Materials and Methods

The test culture. The investigations were carried out with carefully differentiated cultures of *Serratia marcescens* strain ATCC 274, maintained in 0.3 per cent beef extract broth. The cultures were 45 to 48 hr old when used in tests (Kethley *et al.*, 1956).

Chambers and equipment for the production and maintenance of the bacterial aerosol. The organisms were dispersed into the air from 0.3 per cent beef extract broth culture with a no. 40 De Vilbiss[2] all-glass atomizer, classified in a prechamber so that 90 per cent of the particles in the cloud issuing from the prechamber contained only one bacterium, and none contained more than two. This aerosol cloud was then diluted with air of the desired humidity and temperature and introduced into either a cubical or cylindrical chamber system. The production of the test aerosol and the details of the chamber system are described in detail elsewhere (Kethley *et al.*, 1956; Kethley *et al.*, 1957).

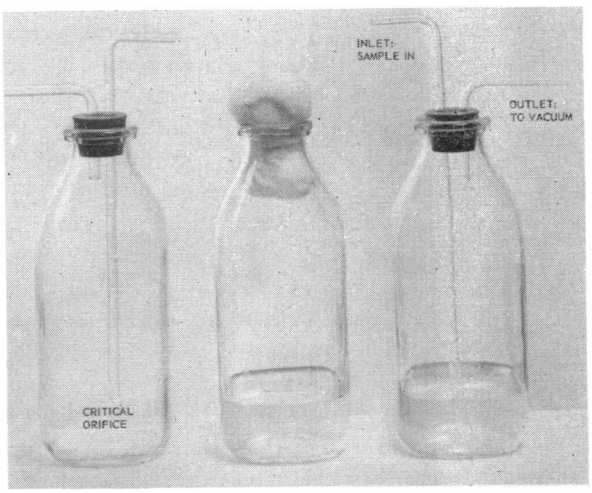

Fig. 1. Critical-orifice liquid impingers

Critical-orifice liquid impinger samplers. (See figure 1.) This sampler functions by the process of passing an air sample through an orifice under such conditions that the critical pressure ratio is maintained across the orifice (Perry, 1941); the air stream strikes a liquid bed, as distinct from those samplers in which the air stream impinges on a solid surface.

The use of the samplers shown in figure 1 has been described previously (Kethley *et al.*, 1956). As shown in this figure the critical orifice can be seen at the end of the inlet tube in the empty sampler on the left. In the center of figure 1 is the milk bottle with sampling fluid prior to sterilization, and on the right is the sampler ready for use.

The critical orifice is actually a descriptive term for an operating condition, that is, the critical pressure ratio exists across the orifice. The critical pressure is that ratio between downstream and upstream pressures required to produce acoustic velocity. Because this velocity is not exceeded by further decreases in the vacuum (downstream pressure), the flow is fixed. For air the critical pressure ratio is 0.53, and if the upstream pressure is 760 mm Hg (30 in), the downstream pressure must be no greater than 402 mm Hg (15.8 in).

[1] These studies were supported in part by National Institutes of Health grant, G-2771.

[2] The De Vilbiss Company, Toledo, Ohio.

Because commercial vacuum gauges are calibrated in inches of vacuum, barometric pressures are converted by subtracting them from 30 in Hg. It must be remembered that the critical pressure ratio refers to the drop across the orifice, and pressure drops in the lines must be considered if the gauge is located at the vacuum source. In practice, a gauge reading of 22 in Hg vacuum is the minimum vacuum acceptable for the maintenance of critical pressure ratios across a bank of samplers. A high volume pump is required for this purpose; the Nash "Hytor"[3] pumps used by us are each capable of maintaining this degree of vacuum for a total of at least 50 L per min. It should be noted that the ordinary small laboratory vacuum pump will handle only 1.0 L per min under similar conditions.

For orifices made by shrinking the tips of soft glass tubing, the tubing having a diameter not less than 5 times that of the resultant orifice, the experimentally determined relation between orifice diameter and volume of flow at acoustic velocity (at standard conditions of temperature and pressure) is approximately:

$$Q = (d^2)\ (9)$$

Where Q is the volume of air in L per min, and d is the diameter of the orifice in mm. Thus an orifice of 0.33-mm diameter will deliver approximately 1.0 L per min at acoustic velocity, or a 3.3-mm diameter orifice will deliver approximately 100 L per min, provided a pump were available to maintain the critical pressure ratio across the orifice.

After tubing tips have been constricted in a flame to form the estimated desired size orifice, these are individually connected to the vacuum line and screened by measuring the intake air with a flowmeter ("Flowrator"[4]). Those orifices having a flow rate 20 per cent in excess of the desired are re-shrunk; those at 20 per cent less than desired are discarded. The selected orifices are then carefully calibrated against a wet-test meter,[5] the meter being positioned upstream from the orifice (that is, not under vacuum).

The liquid impinger media. In addition to the physical aspects of sampling for airborne particulates, studies on bacterial aerosols are complicated by the biologic nature of the experimental material. Media must be adequate not only for collecting the particles, but must also furnish a suitable environment for the living cells collected. For this reason, various bacteriologic media were used in the samplers. These are described in the tables showing the results obtained.

Because of the great amount of frothing resulting from the passage of the air sample through the various media, an antifoaming agent is added to each sampler prior to sterilization, either 0.5 ml medicinal grade olive oil or a loopful of Dow AF[6] to each 200 ml of sampling fluid.

Plating technique. Following sampling, aliquots of the sampler fluid were plated, either directly or diluted, in triplicate. The plating medium consisted of: tryptone glucose agar, 24 g; sodium chloride, 5 g; dibasic sodium phosphate, anhydrous, 2.5 g; distilled, deionized water to make 1 L. Employing this medium, colonies of the test organism were counted after incubation at 35 C for 18 hr.

Photographic technique. In order to distinguish the details of the action occurring during operation of a critical-orifice impinger, it was necessary to make studies on a low rate-of-flow orifice (0.2 L per min), otherwise violent agitation taking place in the liquid completely obscured the details of action. Photographs were made with a Leica[7] camera and a Strobelume[8] light source at 4 microseconds exposure. Also, photographs were taken on 16-mm film at 4000 frames per sec using the Fastax camera.[9]

Results and Discussion

Internal geometry of the sampler and rate of flow through the sampler. Prior studies by Rosebury (1947) show (1) when the tip of the sampler is very close to the bottom of the container, reduced effectiveness of recovery results, and (2) highly variable results are obtained with samplers operating at rates as great as 16 L per min. It was suggested that these two factors are interrelated. In our own work, high speed photographs show a definite stem emerging from the orifice; if the length of this stem increases with increasing rate of flow, the minimum distance required between the tip and the bottom would increase accordingly. It is suggested that the reduced effectiveness of sampling caused by these factors is due to re-atomization of the bacterial particles under conditions of extreme agitation. Studies were carried out with known numbers of bacteria added to the sampling fluid, the tip of the sampler being placed at various distances from the bottom of the container. The results of these studies indicate that when the jet created by passage of air through the orifice at sonic velocity strikes the unyielding surface of the sampler bottom, re-atomization occurs, causing significant losses. Flows at less than sonic velocity did not cause significant losses.

Further verification of the contention that the tip location and flow rate are interrelated in the operation of critical-orifice liquid impingers is to be found in the

[3] Nash.

[4] Fischer and Porter Co., Hatboro, Pennsylvania.

[5] Precision Scientific Co., Chicago, Illinois.

[6] This material is composed of approximately 30 per cent of the antifoaming agent, a chloro-siloxane; 12 to 15 per cent emulsifiers, polyoxyethylene and glycerol monostearates; the balance being water. Dow Corning Corporation, Midland, Michigan.

[7] Ernst Leitz, Wetzler, Germany.

[8] Heiland Research Corp., Denver, Colorado.

[9] Western Electric Co., Inc., New York.

TABLE 1. *Effect of tip position on relative recovery of airborne Serratia marcescens at 20 C, 60 per cent relative humidity; sample containers 1-quart milk bottles with 200 ml gelatin fluid**

Flow Rate of Sampler at Critical Pressure Ratio	Relative Per Cent Recovery‡ of Viable Airborne Organisms	
	Tip 1 cm from bottom of container	Tip 3 to 4 cm from bottom of container
L/min†		
1	100	100
2	90	—
5	60	99
15	—	68
20	—	60
28	38	55

* Medium no. 2, table 2.

† For flow rates from 1 to 10 L per min, orifice diameter ranges from 0.33 mm to 1.1 mm when made from tubing having an internal diameter of 6 mm. For flow rates from 10 to 30 L per min, orifice diameter ranges from 1.1 mm to 1.8 mm when made from tubing having an internal diameter of 9 mm.

‡ Averages of six determinations each, the numbers recorded in the 1 L per min samplers being set to 100.

results in table 1. The data in this table indicate that moving the tip away from the bottom of the container increases the effectiveness of collection by the higher volume samplers.

Tests were carried out with 28 L per min critical orifices in the tall sample container of the Greenberg-Smith[10] impinger in order to increase the distance between the tip and bottom of the container. Compared to the 1 L per min samplers, in the Greenberg-Smith container: with the tip 4.0 cm from the bottom, 34 per cent recovery; tip 8.5 cm from the bottom, 50 per cent recovery. It is to be noted that the Greenberg-Smith container is 5.5 cm in diameter, whereas the milk bottles are square in cross section, 8.5 cm on a side. This difference in cross-sectional dimensions is offered as an explanation for the low recoveries.

Viability of the organisms in the sampler. The results of experiments in which known numbers of organisms were introduced into the samplers operated critically for flow rates of 1 or 5 L per min for a period of 1 hr indicated that the sampling process has no hostile effect on the organisms studied. The tips were positioned 3 to 4 cm from the bottom of the milk bottle containers. No significant change in numbers of viables was found during the course of the experiment.

Effect of the collecting media. The comparative intersampler performance of the gelatin solution using the 1.0 L per min critical-orifice impingers was very satisfactory. However, the question arose as to whether or not this medium, because of its chemical and/or physical composition, was actually demonstrating all of the potentially viable cells collected from a bacterial aerosol cloud. The results shown in table 2 comparing

[10] Corning Glass Works, Corning, New York.

TABLE 2. *Effect of various impinger media on collection of airborne Serratia marcescens at 20 C, 74 per cent relative humidity*

No.	Medium*	Antifoam†	Avg Relative Recovery, Counts Brain-Heart Samplers Set to 100
1	Gelatin	(Dow AF)	42.5
2	Gelatin	(Oil)	42.5
3	Gelatin-broth	(Dow AF)	70.5
4	Gelatin-broth	(Oil)	60.0
5	Broth	(Dow-AF)	70.5
6	Broth	(Oil)	46.0
7	Gelatin-brain-heart	(Dow AF)	100.0
8	Gelatin-lactose	(Oil)	73.0
9	Gelatin-proteose peptone-yeast extract	(Oil)	59.0
10	Gelatin-NaCl-lactose	(Oil)	69.0
11	Gelatin-proteose peptone-NaCl-yeast extract	(Oil)	104.5

* Composition of media:

1 and 2—0.2 per cent gelatin solution (Pharmagel A—Pharmagel Corporation, New York, New York), Na_2HPO_4, anhydrous, 0.08 g per g of gelatin; pH 6.95.

3 and 4—0.2 per cent Pharmagel A (buffered) in 0.3 per cent beef-extract broth; pH 6.98.

5 and 6—0.3 per cent beef-extract broth; pH 6.8.

7—0.2 per cent Pharmagel A (buffered) in 3.7 per cent brain-heart infusion; pH 7.2.

8—0.2 per cent Pharmagel A (buffered), 0.25 per cent NaCl, 0.5 per cent lactose; pH 6.65.

9—0.2 per cent Pharmagel A (buffered), 2 per cent proteose peptone, 0.3 per cent yeast extract, 0.25 per cent NaCl; pH adjusted with potassium hydroxide (10 per cent) to pH 6.9.

† Antifoams: Dow AF—loop inoculum per impinger sample; oil-olive oil—0.5 ml per impinger sample.

the collecting ability of a series of impinger media of different compositions suggest that many of the injured cells within the group of lower viability potential were previously lost because of the absence, in the collecting medium, of suitable nutrient constituents of such nature as to reverse or terminate the injury mechanism.

A comparison of the use of plain gelatin fluid and the fluid enriched with brain-heart infusion showed that the enriched fluid demonstrated 50 per cent more viables. The significance of the difference in efficiency of sampling between the two media in estimating particle size has already been indicated (Kethley *et al.*, 1957). Enrichment of the plating media did not produce any significant differences in total count.

Addition of antifoam agent to impinger media. Concomitant with the studies on the effect of various impinger media on the collection of airborne organisms, comparative studies were made on the addition of Dow AF[6] (a dispersion of Dow Corning Antifoam A silicone defoamer which can be dilated in water) and olive oil to the impinger media as antifoaming agents.

Results of this study showed that there is no significant difference in the numbers of organisms collected. The Dow AF antifoaming agent visually appears to be more effective in dampening foam formation. Also, it does not produce objectionable effects in the solid plating media as do the oil globules.

Physical characteristics of sampler fluids. The action of the orifice-type liquid impinger is also dependent upon the inherent properties and condition of the liquid medium itself. From the standpoint of rigidity and adhesiveness of the collecting surface, the liquid should be of such composition as to resist deformation by the orifice jet and yet at the same time possess characteristics which enable the liquid to wet and entrap the airborne particles. This suggests a liquid with a comparatively high viscosity and low surface tension. Limited observations and measurements were made on some of the physical characteristics of the sampler fluids. Surface tension values obtained for the more complex fluids were lower than that for the plain gelatin fluid. Also, the effect of the emulsifying agent (resulting from addition of Dow AF and filtering) in depressing the surface tension was evident for all fluids, but less so with the plain gelatin. This may be another clue to the enhanced ability of the more complex fluids in collecting airborne organisms, in addition to any biologic effect. Slightly higher viscosity values were obtained for the more complex fluids which may further explain their greater effectiveness in collecting airborne organisms. Further investigation of these factors might lead to the development of a fluid which would overcome the reduced effectiveness occasioned by the use of orifices having flow rates in excess of 1.0 L per min.

Temperature of sampler. There has been some indication that the internal temperature of the sampler during the operating period may affect the collecting ability of the sampler. Factors such as viscosity, surface tension, and general gas solubility are somewhat dependent upon temperature. In order to study the possible effect of temperature on the sampler fluid, as distinct from any effect of temperature upon the viability of the organisms in the airborne state, the aerosol chamber system was operated at a constant temperature and humidity, and the samplers were individually heated or cooled to various temperatures. The results of this investigation are shown in table 3, both for organisms at the inlet of the chamber and at the outlet from the chamber.

The information in table 3 is interpreted to indicate that for critical-orifice liquid impingers, temperatures of 10 to 20 C exert a uniform effect upon the sampler, but that at temperatures of 32 C or above, a lowered effectiveness is caused by the temperature increase. It is not known whether this is the result of changes in physical factors, or whether it is a biologic response, or some combination of both.

TABLE 3. *Effect of temperature of the impinger fluid* on the number of viable airborne organisms demonstrated. Room and chamber air maintained at 20 C, 60 per cent relative humidity*

Temperature of Fluid in Sampler	Indicated Concentration of Viables per L of Air					
	Inlet of chamber			Outlet from chamber		
	Avg	n†	S.E.	Avg	n	S.E.
C						
10	9,000	12	570	5,700	11	240
13.5	8,900	3	870	5,200	3	270
20	10,500	6	650	5,500	6	280
32	7,400	6	800	4,000	6	290
40	4,000	6	660	1,900	6	390

* Brain-heart gelatin fluid (medium no. 7, table 2, 1.6 per cent brain-heart infusion instead of 3.7) 200 ml in quart milk bottles, 1.0 L per min critical orifice 3 to 4 cm from bottom of container.

† n = number of replications.

Various other factors. Employing 1.0 L per min critical-orifice liquid impingers, 1-quart milk bottles containing 200 ml of brain-heart fluid, the tip positioned just below the surface of the liquid (3 to 4 cm from the bottom of the container), the effects of the following variables were investigated at 20 C:

Particle size and relative humidity: The good agreement between calculated and determined sizes for particles ranging in size between 0.6 and 3.0 μ in diameter under various conditions of relative humidity at 20 C (Kethley *et al.*, 1957), indicates the effectiveness of the samplers; the particle size determinations being made from a comparison of aerial concentrations and numbers settling per min.

Sampling periods: Comparison of results obtained from 1-min periods of sampling to those from 30-min periods indicated no significant difference due to length of sampling period.

Concentration of the bacterial aerosol: These samplers have proven satisfactory in sampling bacterial aerosols varying in concentration from 500 to 500,000 per L, the results showing good correlation to the numbers of bacteria atomized. Direct studies where identical cultures were atomized both undiluted and diluted 1:10 with broth yielded aerial concentrations of 50,000 and 5000 per L, respectively.

Analysis of high-speed photographs; suggested mechanism of action of the impingers. Apparently, the high velocity of the air stream results in the formation of a definite jet or stem at the exit of the orifice as indicated in figure 2. This jet is oscillating and sometimes a bubble is formed directly at the tip of the stem, and other times it simply breaks into numerous smaller bubbles. It is a combination of the jet formation and large and small bubble generation that seems to account for the

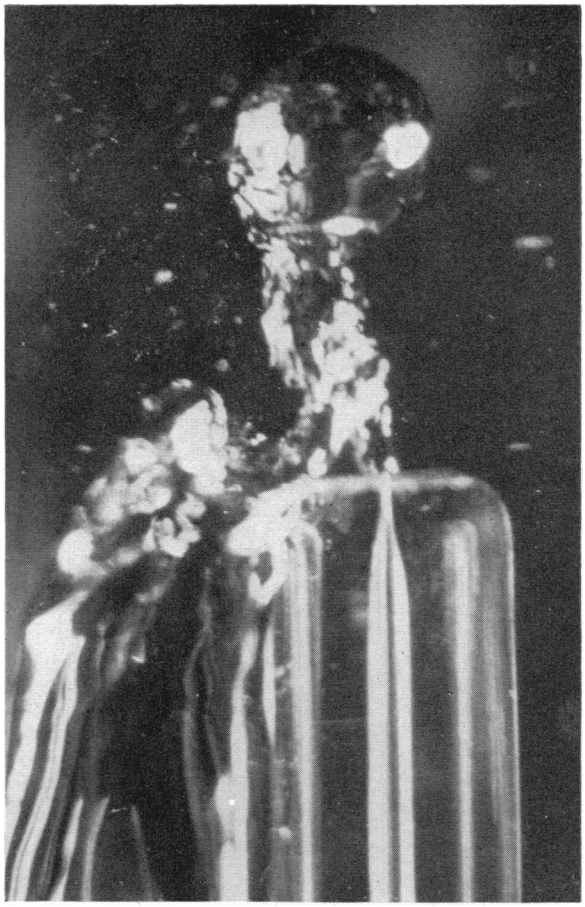

FIG. 2. High-speed photograph of 0.2 L per min critical-orifice liquid impinger in operation.

ability of the critical-orifice liquid impinger to remove particles from the air stream. Considering the results of the studies on internal geometry, it is assumed that re-atomization occurs whenever this jet strikes a solid surface.

In conjunction with the rate of flow through the impinger orifice, the configuration and size of the jet may affect the action occurring at the contact surface of the jet stream and liquid. It is reasonable to believe there may be advantages in using the smaller diameter orifices. If the concentration of particles per unit volume is assumed constant, a small jet stream may increase the probability of immediate contact occurring for a given surface contact area. [For further consideration of these factors, reference is made to the excellent review by Haney (1954), and to the original articles cited in that review.]

Efficiency of the samplers for bacterial aerosols. It is virtually impossible to determine the absolute efficiency of the critical-orifice liquid impinger under conditions of operation. By collecting on cotton the spores which passed through the impingers, Rosebury (1947) estimated the efficiency of the impinger as greater than 95 per cent. A comparative study by Kluyver and Visser (1950) showed Rosebury's capillary impinger to be more efficient than bubbler samplers, and estimated that the capillary impinger collected more than 99 per cent of airborne spores. In a recent comparative study, Orr *et al.* (1956) have shown the value of a thermal precipitator developed by this group (Kethley *et al.*, 1952) for aerobacteriologic studies; their results also indicate a high efficiency for the critical-orifice liquid impingers. Although the thermal precipitator is considered as essentially 100 per cent efficient, the limitations of a solid surface sampler and the ability to aliquot a liquid sample prompted us to explore further the use of liquid impingers. The results of a limited number of comparative studies were so unfavorable to other samplers tested (sieve-samplers, Greenberg-Smith and midget impingers) that no further attempts were made to use routinely any sampler other than the 1.0 L per min critical-orifice liquid impinger (Kethley *et al.*, 1956). The most convincing proof of the very high efficiency of these samplers results from studies showing close agreement between calculated and determined particle diameters of bacterial aerosols in which the air samples were taken with critical-orifice liquid impingers (Kethley *et al.*, 1957). Any efficiency significantly less than 100 per cent would have yielded determined values of particle diameters larger than the calculated; the method of determination involving a comparison of the aerial concentration and the numbers settling per min.

SUMMARY

The effect of a number of factors has been determined for the critical-orifice liquid impinger in the sampling of bacterial aerosols: internal geometry and rate of flow through the sampler; composition of the collecting fluid; temperature of the sampler; concentration of the bacterial aerosol. It has been shown that, properly designed and used, the critical-orifice liquid impinger exhibits a high degree of effectiveness in sampling bacterial aerosols. The most effective critical-orifice liquid impinger for this purpose is the 1.0 L/min in a 1-quart milk bottle containing 200 ml of gelatin fluid enriched with brain-heart infusion, the tip containing the orifice being immersed beneath the surface of the liquid, but not less than 2 cm from the bottom of the container. Under ordinary conditions, the effectiveness of such a sampler is adversely affected only by temperature, being reduced at temperatures as high as 32 C. The reproducibility, ease of use and manufacture of these samplers commend their employment in the collection of airborne bacteria under a variety of conditions of particle size, relative humidity, temperature, periods of sampling, and concentrations of the airborne bacteria.

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Multiple Factor Experimentation with Reference to Biosynthetic Yield of Aterrimin[1]

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The common problem of improving biosynthetic yields has usually been met by investigating factors one at a time while holding the others constant. Experimental designs that allow a number of factors to be varied simultaneously and the effect of each factor to be calculated from the results, have been recognized in many fields to have important advantages (Bennett and Franklin, 1954; Cochran and Cox, 1950; Davies, 1954; Finney, 1955; Gore, 1950, 1951). An application to penicillin yield improvement in pilot-plant studies has been described by Brownlee (1950, 1953). There can be little doubt as to the value of such designs in the phase of biosynthetic yield improvement wherein preliminary investigations have disclosed important factors, the optimal combination of these factors must be approximated, and experimentation is expensive, as it usually is where fermentors must be used.

The initial phase of biosynthetic yield improvement, however, involves a search for the more favorable conditions and a rough recognition of the more critical and the less critical factors. A peculiarity of the problem lies in the tremendous variety of choices of potentially important factors, nowhere more evident than in strain selection where possibly hundreds or even thousands of strains must be tested. Also, the necessity for preoccupation with the higher yields (that is, with the best available approximations of optimal levels or choices of the more important factors) virtually forces a sequential type of experimentation. Success in the initial phase of the investigation is measured not so much by a detailed knowledge of the interaction of factors as by the finding of one or a few combinations of factors that give encouraging yields.

The initial phases of investigations are the more difficult to formalize, and are usually dismissed in the handbooks of experimental design by such phrases as "preliminary experimentation" (meaning "nonstatistically designed experimentation"), "knowledge, experience and ingenuity of the investigator," and so forth. Indeed, one may suggest that a preoccupation with statistical design in this phase is hazardous if interest is diverted from consideration of the wide range of biologic and biochemical factors that may affect biosynthetic yields. However, we believe that some of the advantages of multiple-factor experimentation may be had in preliminary investigation of biosynthetic yields without resort to statistical design of experiments, the risks being essentially those inherent in the classical one-factor-at-a-time approach whether the latter is done under statistical control or not. It is also to be hoped that more investigators of biosynthetic-yield improvement will try statistical designs and that investigators of experimental design will make proposals better adapted to relatively brief surveys of very large numbers of variables.

In an exploration of this problem we have allocated choices or levels of various factors formally according to an array of the latin square type, ignored unobvious interactions between variables, and omitted estimates of significance in the interest of covering the widest possible ground in the time available. We suggest that

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