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Staff Meeting Report

Distribution of Blood Flow to the Tissues of The Small Intestine of the Dog*

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The blood flow through an organ may usually be measured by the simple procedure of inserting a cannula into the vein draining the organ and collecting a timed sample of the outflow. The determination of the *proportion* of the total flow that passes through the different tissues that make up the organ, however, is seldom amenable to this approach. This report describes a new technique that promises: (1) to permit an estimation of this regional distribution of flow, and (2) to provide information concerning the relative flows through arteriovenous (A-V) channels of different sizes in each tissue.

The method was developed during experimentation, utilizing a modification of the technique of Prinzmetal *et al.*[1] to determine the blood flow through A-V anastomoses in the small intestine. In these experiments a known number of glass microspheres small enough to pass through the A-V anastomoses but too large to pass through capillaries was injected into the artery of a segment of the organ. The venous outflow was collected and the spheres therein were counted microscopically. With the reasonable assumption that the spheres are distributed at arterial bifurcations in the same proportion as is the blood, the fraction of injected spheres recovered was taken as a measure of the fraction of the arterial blood that had passed through the A-V anastomoses. The spheres, which were obtained from the Minnesota Mining and Manufacturing Company, were received as a mixture with diameters varying from 5 to 100 microns. For use in the experiments described they were fractionated into homogeneous samples by a series of multiple elutriations and timed decantations.

To study the organ *in situ* it was necessary to find a method for

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decolorizing the venous blood in order to see and count the microspheres under the microscope. Decolorizing proved very difficult to accomplish without losing some of the spheres. In attempting to solve this problem, we reasoned that if the spheres were made radioactive, a simple determination of the radioactivity of the venous blood would provide the information necessary to calculate the proportion of the injected spheres that passed through the A-V shunts. Therefore the spheres were subjected to neutron bombardment in the atomic pile at the Oak Ridge National Laboratory.

The glass of which these spheres were made contained about 10 per cent NA_2O by weight, and a part of this ordinary Sodium-23 was converted by neutron capture into radioactive Sodium-24.

Sodium-24 emits large quantities of gamma rays; consequently its content may be measured in blood or tissue without concern for self-absorption. Thus it immediately became apparent that the number of spheres in any solid tissue as well as in blood could be determined by the simple procedure of dissecting out the tissue and measuring its radioactivity. Since those spheres that remained behind in any particular tissue presumably did so because they lodged in vessels smaller than the diameter of the spheres, the number of spheres recovered from that tissue provided a measure of the flow through vessels smaller than the spheres. If the spheres were small enough to pass through all A-V channels except capillaries, the radioactivity of the tissue was a measure of the tissue capillary flow. Thus, the technique permitted the estimation of the relative capillary blood flows in the different tissues of a given organ.

The method employed in the present study can be briefly summarized as follows:

(1) A small group of the irradiated spheres was suspended in a vial of 1½ per cent gelatin solution. The mixture was stirred to keep the spheres in suspension.

(2) With a micrometer syringe, 10 or 20 microliters of the suspension were drawn into a short section of polyethylene tubing, the concentration of spheres being such that this volume contained no more than one thousand spheres.

(3) Through a 27-gauge needle tip on the end of the polyethylene tubing these spheres were injected into the artery of an isolated loop of small intestine.

(4) The venous outflow was collected for two minutes by means of a cannula in the single vein draining the loop.

(5) The loop was removed from the animal and separated quan-

titatively into four tissue fractions: mucosa, submucosa, muscle, and mesentery.

(6) The radioactivity of the blood and of each tissue sample was determined in a conventional well-type scintillation detector.

(7) The fraction of the total radioactivity recovered in each tissue was calculated, this being equivalent to the fraction of the injected spheres in the same tissues.

Table 1 shows the fractional distribution of spheres of three different sizes in the tissues of jejunal loops of dogs which had fasted for 24 hours. The diameters of spheres listed are mean values with a standard deviation of about 1.5 μ. These data provided information not only about the relative blood flows through the tissues but also about the size of the A-V channels. Only 1 per cent of the injected radioactive material was found in the venous blood when 43μ-spheres were injected. Since a part, if not all, of the material in the venous blood was radiosodium washed out of the spheres, we concluded that all A-V channels are less than 43 μ in diameter. Since only 4 per cent of the 20 μ spheres passed through into the venous outflow, 96 per cent of the blood supply to jejunum in a fasted state flowed through A-V channels that were less than 20 μ in diameter; therefore the A-V anastomotic flow is quite small. With 12 μ spheres, 28 per cent passed through; thus, 24 per cent of the blood flowed through A-V channels that were less than 20 μ but no more than 12 μ in diameter. This is the range of size of the A-V bridges of Zweifach,[2] and this flow is referred to here as A-V bridge flow. The fractions of 20 μ and 12 μ spheres recovered from muscle and mesentery were nearly identical, which means that most of A-V bridge flow is in the mucosa and submucosa. Most of the 43 μ spheres lodged in the submucosa, very few reaching the mucosa; the arteries carrying blood from submucosa to the mucosa apparently were less than 43 μ in diameter. Since the fractions of spheres of all sizes recovered from the muscle were about the same, the submucosa-to-muscle arteries were at least 43 μ in diameter.

TABLE 1
FRACTIONAL DISTRIBUTION OF SPHERES
IN THE TISSUES OF FASTED JEJUNUM

Sphere diam.	No. of expts.	Fraction of spheres recovered				
		Mucosa	Submucosa	Muscle	Mesentery	Venous blood
43 μ	2	.03	.61	.16	.19	.01
20 μ	15	.53	.18	.15	.10	.04
12 μ	6	.39	.07	.14	.12	.28

The mean total blood flow through jejunum in a fasted state was measured as 57 cc/minute/100 gm. wet tissue. The distribution of this total flow to A-V channels of different sizes is shown in Table 2.

TABLE 2
DISTRIBUTION OF BLOOD FLOW TO A-V CHANNELS OF DIFFERENT SIZES IN FASTED JEJUNUM

Total blood flow per 100 gm.	=	57.0 cc/min	(100%)
A-V anastomotic flow (>20μ)	=	2.3 cc/min	(4%)
A-V bridge flow (12 to 20μ)	=	13.7 cc/min	(24%)
Capillary flow (<12μ)	=	41.0 cc/min	(72%)

TABLE 3
DISTRIBUTION OF CAPILLARY BLOOD FLOW AMONG THE DIFFERENT TISSUES OF FASTED JEJUNUM

Mucosa	=	22.2 cc/min	(54%)
Submucosa	=	4.0 cc/min	(10%)
Muscle	=	8.0 cc/min	(19%)
Mesentery	=	6.8 cc/min	(17%)

Table 3 depicts the distribution of the total capillary flow (41 cc/minute/100 gm.) among the different tissues is shown. The dis tribution of the total A-V bridge flow (13.7 cc/minute/100 gm.) is shown in Table 4.

TABLE 4
DISTRIBUTION OF A-V BRIDGE BLOOD FLOW AMONG THE DIFFERENT TISSUES OF FASTED JEJUNUM

Mucosa	=	7.3 cc/min	(54%)
Submucosa	=	5.8 cc/min	(42%)
Muscle	=	0.6 cc/min	(4%)
Mesentery	=	0.0 cc/min	(0%)

The relative weights of the different tissues of the small intestine were determined to permit the calculation of blood flow per unit weight of tissue. In Tables 5 and 6, the capillary and capillary plus A-V bridge flows per 100 gm. tissue are shown for the four tissues.

Figure 1 schematically summarizes the distribution of blood flow to A-V channels of different sizes in the different tissues of the canine jejunum in a fasted state.

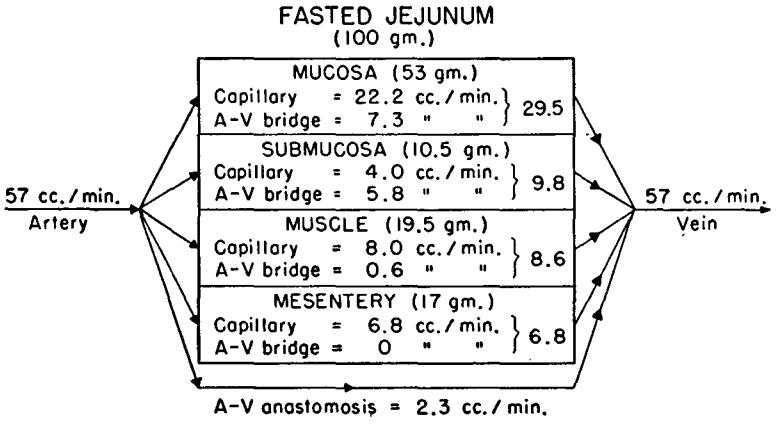

Fig. 1. Circulation schema for 100 gm. of canine fasted jejunum.

Experiments were also performed with loops of ileum in the same fasting dogs. The fractions of spheres recovered from the different tissues are shown in Table 7. When these data along with the calculated flow data of Tables 8 and 9 were compared with the results

TABLE 5

CAPILLARY BLOOD FLOWS IN FASTED JEJUNUM PER 100 GM. OF:

Mucosa	= 42 cc/min
Submucosa	= 41 cc/min
Muscle	= 39 cc/min
Mesentery	= 41 cc/min

TABLE 6

CAPILLARY + A-V BRIDGE FLOWS IN FASTED JEJUNUM PER 100 GM. OF:

Mucosa	= 56 cc/min
Submucosa	= 93 cc/min
Muscle	= 44 cc/min
Mesentery	= 41 cc/min

TABLE 7

FRACTIONAL DISTRIBUTION OF SPHERES IN THE TISSUES OF FASTED ILEUM

Sphere diam.	No. of expts.	Fraction of spheres recovered				
		Mucosa	Submucosa	Muscle	Mesentery	Venous blood
43 μ	1	.00	.56	.24	.19	.01
20 μ	18	.32	.23	.27	.15	.03
12 μ	9	.38	.08	.22	.15	.17

TABLE 8
DISTRIBUTION OF BLOOD FLOW TO A-V CHANNELS OF DIFFERENT SIZES IN FASTED ILEUM

Total blood flow per 100 gm.	=	59.0 cc/min	(100%)
A-V anastomotic flow ($>20\mu$)	=	1.8 cc/min	(3%)
A-V bridge flow (12 to 20μ)	=	8.2 cc/min	(14%)
Capillary flow ($<12\mu$)	=	49.0 cc/min	(83%)

TABLE 9
CAPILLARY BLOOD FLOWS IN FASTED ILEUM PER 100 GM. OF:

Mucosa	=	49 cc/min
Submucosa	=	38 cc/min
Muscle	=	55 cc/min
Mesentery	=	49 cc/min

of the experiments on jejunal loops several interesting points emerged: The total blood flow per 100 gm. of ileum was about the same as in the jejunum. The A-V anastomotic flow was quite small as in the case of the jejunum. Ileal A-V bridge flow was somewhat lower. The capillary blood flows per unit weight of tissue appeared to be slightly greater for muscle, mucosa, and mesentery, but the significance of the difference, particularly in the case of the latter two, was questionable. The distribution of capillary blood flow in tissues of both jejunum and ileum does not appear markedly disproportionate to the weights of the tissues.

Unlike the experiments on jejunal loops, the studies on the ileum revealed a greater recovery of 12 μ spheres than of 20 μ spheres from mucosa. The most plausible explanation is that some of the arteries carrying blood from ileal submucosa are less than 20 μ in diameter. It should also be noted that about one-third of the A-V bridge flow was localized in the muscle.

In order to determine if tissue capillary blood flow in the small intestine is affected by the digestive state, experimental dogs were fed a meat meal at six hours and again at two hours before the experiment. The data obtained from experiments with jejunal loops in these fed animals are shown in Tables 10, 11, and 12. The total A-V anastomotic, A-V bridge, and capillary flows were essentially the same as they were in the fasting animals. The major effect of feeding seemed to be to divert capillary flow from mesentery to the other three tissues in about equal proportion. One other difference is that the sub-

mucosa-to-mucosa supply arteries seem to have constricted as fewer 20 μ spheres entered the mucosa.

TABLE 10
FRACTIONAL DISTRIBUTION OF SPHERES
IN THE TISSUES OF FED JEJUNUM

Sphere diam.	No. of expts.	Fraction of spheres recovered				
		Mucosa	Submucosa	Muscle	Mesentery	Venous blood
43 μ	2	.02	.64	.26	.08	.00
20 μ	14	.28	.29	.19	.20	.04
12 μ	6	.46	.10	.15	.06	.23

TABLE 11
DISTRIBUTION OF BLOOD FLOW TO A-V CHANNELS OF DIFFERENT SIZES
IN FED JEJUNUM

Total blood flow per 100 gm.	= 55.0 cc/min	(100%)
A-V anastomotic flow ($>20\mu$)	= 2.2 cc/min	(4%)
A-V bridge flow (12 to 20μ)	= 10.5 cc/min	(19%)
Capillary flow ($<12\mu$)	= 42.3 cc/min	(77%)

TABLE 12
CAPILLARY BLOOD FLOWS IN FED JEJUNUM PER 100 GM. OF:

Mucosa	= 48 cc/min
Submucosa	= 52 cc/min
Muscle	= 48 cc/min
Mesentery	= 16 cc/min

The data obtained from experiments with ileal loops in the same fed animals are shown in Tables 13, 14, and 15. Feeding had several marked effects on the pattern of circulation in this part of the intestine: The total flow was significantly elevated (+31%). The A-V anastomotic flow was unchanged, but the A-V bridge flow was more than doubled. The capillary blood flow through the muscle increased significantly, but through other tissues this flow was little affected.

TABLE 13
FRACTIONAL DISTRIBUTION OF SPHERES IN THE TISSUES OF FED ILEUM

Sphere diam.	No. of expts.	Fraction of spheres recovered				
		Mucosa	Submucosa	Muscle	Mesentery	Venous blood
43 μ	3	.02	.50	.29	.18	.01
20 μ	13	.29	.27	.26	.16	.02
12 μ	9	.28	.07	.27	.09	.28

TABLE 14
DISTRIBUTION OF BLOOD FLOW TO A-V CHANNELS OF DIFFERENT SIZES IN FED ILEUM

Total blood flow per 100 gm.	= 77.0 cc/min	(100%)
A-V anastomotic flow (>20μ)	= 1.5 cc/min	(2%)
A-V bridge flow (12 to 20μ)	= 20.0 cc/min	(26%)
Capillary flow (<12μ)	= 55.5 cc/min	(72%)

TABLE 15
CAPILLARY BLOOD FLOWS IN FED ILEUM PER 100 GM. OF:

Mucosa	= 48 cc/min
Submucosa	= 46 cc/min
Muscle	= 89 cc/min
Mesentery	= 34 cc/min

In summary, the capillary flows per unit weight of mucosa, submucosa, muscle, and mesentery in fasted jejunum and ileum were about the same (40-50 cc/minute/100 gm.), with the possible exception of the flow through the ileal muscle, which was slightly higher (55 cc/minute/100 gm.) Feeding caused a slight increase in capillary flow through mucosa, submucosa, and muscle at the expense of the mesentery in the jejunum without causing any significant change in total flow or in the distribution of the flow to the A-V channels of different sizes. In the ileum, feeding caused an increase in the total blood flow, most of which passed through the muscle capillaries and the A-V bridges; little change occurred in the capillary flows of the other tissues. Arteriovenous anastomotic flow was in all instances very low (2 to 4 per cent).

We believe that the technique used offers a new tool for the study of what might be called the functional anatomy of the vascular system of an organ.

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Staff Meeting Report

Genetics of Antibiotic-Producing Streptomycetes*†

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Introduction

Microbial genetics became established as a distinct field of study when the mold *Neurospora crassa* began to compete with the fruit fly, *Drosophila melanogaster*, as the preferred organism for genetic studies. Researches with *N. crassa* have served as a bridge between biochemistry and genetics; the elucidation of tryptophan synthesis remains a noteworthy example for the application of genetics to biochemistry.

Wild-type strains of *N. crassa* can synthesize tryptophan from building blocks provided by the catabolism of carbohydrates; however, a mutant strain of *N. crassa* isolated by Mitchell and Lein,[1] required tryptophan but could not use its precursor, indol, for growth. Genetic analyses demonstrated that this change involved a single genetic locus, whereas biochemical analyses revealed that the mutant lacked the enzyme tryptophan desmolase, which is responsible for the synthesis of tryptophan from indol and serine.[1] In this way the relationship between gene and enzyme was established.

Additional mutants of *N. crassa* which required added tryptophan for growth were isolated, including the following strains: one that would grow only with tryptophan; another that would grow with either tryptophan or indol; a third that would grow with tryptophan, indol, or anthranilic acid; and a fourth strain which would grow with tryptophan, indol, anthranilic acid, or kynurenine.[2] On the basis of this information, the following pathway has been devised: kynurenine → anthranilic acid → indol → tryptophan.

The successor to *N. crassa* has been *Escherichia coli* strain K-12, which has been found to possess a recombinational mechanism.[3] In the course of genetic studies, it was discovered that strain K-12 was lysogenic, i. e., carried a temperate phage, Lambda. Mutants of *E.*

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coli were then obtained which were sensitive, resistant, or lysogenic, with respect to Lambda. When crosses were made between pairs of these mutants, it was demonstrated that the temperate phage behaved as a Mendelian gene and was closely linked to one of the genes controlling galactose fermentation. The relationship between gene and virus was thus established.[4] Following this lead, Lederberg[5] observed that induced Lambda was able to carry genetic information from the disrupted donor cell to an intact recipient cell.[5] These findings have greatly excited workers in cancer biology.

Microbial genetics has contributed not only to related fields of knowledge but also to classical genetics. Current concepts of the role of deoxyribonucleic acid in heredity have been deduced from studies with pneumococcal transformation and salmonella transduction; our concepts of the fine structure of chromosomes and genes have been greatly influenced by work with *E. coli* and bacteriophages. Microbial genetics is expected to affect the course of cancer biology, immunology, morphogenesis, and ecology, among other fields.

Microbial geneticists strive to explore new aspects of genetics by accepted methods, and introduce new methods and organisms, so that new mechanisms may be detected and unsuspected correlations may be recognized. With this hope, the streptomycetes have been added to the expanding list of microbes that are being analyzed genetically.

The Streptomycetes

These filamentous organisms may be phylogenetically related to the true molds,[6] but they are classified more accurately with the bacteria because of similarities in size, chemical composition, physiology, and host-parasite interactions. For example, the diameters of the hyphae of streptomycetes, which range from 0.5 to 2 μ, are much thinner than those of true molds.[7] Moreover, cell walls of streptomycetes are dissolved by the enzyme lysozyme, as are cell walls of several true bacteria;[8,9,10] and furthermore, the cell walls of many streptomycetes contain alpha, epsilon-diaminopimelic acid, an amino acid found in the cell walls of several true bacteria but absent in molds.[11,12] In addition, the streptomycetes are sensitive to several antibacterial antibiotics, including streptomycin, penicillin, bacitracin, and the tetracyclines, but are not inhibited by antifungal antibiotics such as nystatin and cycloheximide.[13] Finally, streptomycetes are attacked by viruses which resemble bacteriophages;[14,15] as yet, investigators have not encountered any virus that is able to attack a true mold, even though ample opportunities have been provided in open

fermentors employing *Aspergillus, Penicillium,* and *Saccharomyces.*

Several streptomycetes are readily cultured from soil samples on a laboratory medium containing only sugar and inorganic salts.[16,17] These organisms, however, are not restricted to saprophytic life, for some are parasitic as well; *Streptomyces scabies,* for example, is a well-known pathogen for potatoes.[18] Moreover, *Nocardia paraguayensis* and *N. maduræ,* which are accepted etiologic agents of mycetoma in man,[19] are best considered as members of the genus *Streptomyces.*[20,21,22] The researches of the past 15 years have de-emphasized the role of these microbes in the soil, and even their importance as etiologic agents, stressing instead their ability to produce clinically useful antibiotics.[23]

Importance of Genetic Studies

Genetic studies on streptomycetes have immediate practical implications for the commercial producers of antibiotics who strive to obtain higher yields more quickly and more efficiently.[24,25] The fermentation industry needs stable strains that not only are resistant to sporadically encountered inhibitors but also are not seriously affected by minor variations in the nutritive substrate. Moreover, strains may be sought which produce less toxic, more active, and more soluble forms of a particular antibiotic.[26] In addition to their practical applications, the streptomycetes merit extensive genetic investigation because they represent excellent tools for fundamental biological research in such areas as: host-parasite interactions; nucleo-cytoplasmic interactions; interactions among diverse nuclei; recombinational processes at various cellular and subcellular levels.

Premises of Genetic Research

Genetic analyses depend upon the stability of recognizable expressions (phenotypes) in a constant environment. In order to investigate many aspects of genetic behavior, however, variants must be obtained. Because suitable mutants arise infrequently, various methods have been devised to increase the rate at which these variants develop, and to increase the efficiency of detecting them once they are formed. Several agents (mutagens) are known to increase the number of mutant streptomycetes formed per cell per generation;[27] among these, ultraviolet irradiation is not only very effective but in addition requires little special equipment and is easily controlled as to dosage.[28]

Even after treatment with a mutagen, the fraction of variants among the wild type population is low. Morphological mutants, of

course, may be detected directly, but in order to detect nutritional variants, additional testing is necessary. Replica plating greatly facilitates the screening of large numbers of colonies for growth-factor requirements.[29] In this way, strains of *Streptomyces* requiring added amino acids, purines, and pyrimidines have been isolated.[30] Certain variants suitable for genetic studies can be obtained without recourse to mutagens; for example, mutants resistant to actinophages and to antibiotics such as streptomycin can be selected directly by plating large numbers of the sensitive wild type population on appropriate media with ample amounts of the inhibitor.[31]

For maximal utility, variants used for genetic studies must have certain characteristics: they must be stable; they should be easily recognized when contrasted to the wild type and to other variants; they should grow at rates comparable to the wild types; some of them should be susceptible to adverse or preferential selection.[32] Three classes of genetic markers that often satisfy all these criteria have been indicated above; namely, drug sensitivity, actinophage susceptibility, and nutritional requirements.

Interactions Between Cells

Various types of interactions between intact streptomycete cells have been recognized.[33] In some instances two growth-factor dependent strains, each of which requires a different amino acid, can grow together even though both substances are absent. It can be proved that the observed interaction is between intact cells because: a) actual contact between the two organisms is not necessary, and b) colonies derived from a single cell resemble only one or the other parent. Such synergism or syntrophism has been demonstrated for several species of *Streptomyces*.[13]

Interactions Within Cells

Intimate interactions have been demonstrated which involve actual cell fusion and thereby bring nuclei of diverse origin together within a common cytoplasmic field.[30] In the simplest process, i. e., heterokaryosis, the parental nuclei maintain their integrity; therefore only three phenotypes can be found: these are the heterokaryotic phenotype and the two parental types which appear as segregants. Complications have been observed, however, as when certain of the isolated segregants have displayed at least one nutritional requirement of each doubly auxotrophic parent. These "anomalous auxotrophic heterokaryons" produced parental segregants during serial subculture. Anomalous, auxotrophic heterokaryons which exhibited two nutri-

tional requirements of one parent and one nutritional requirement from a second parent invariably yielded more segregants resembling the first parent than resembling the second parent.[34] Similar behavior has been studied in *Neurospora crassa,* wherein a heterokaryon which contained more nuclei from parent A than from parent B displayed some expressions typical of parent A and some typical of the normal or balanced heterokaryon.[35,36] It was observed that nuclear ratios, once established, tended to be stable but that during sporulation new ratios could be formed.

A second complication is that a few segregant streptomycetes have exhibited nutritional requirements not recognized in either parent. These segregants could have arisen as spontaneous mutants uncovered because of some nonrandom factor such as selection. Alternatively, the heterokaryotic cytoplasm which was formed by the admixture of two diverse cytoplasms, might have induced genic instabilities in the parental nuclei.[37]

The Status of Genic Recombination

Since nuclear fusion can conceivably occur within heterokaryons, many new phenotypes may be recovered from the descendants of a single cell if recombination occurs during mitosis, or if the number of chromosomes is subsequently reduced to the number found in the parental nuclei.[38] Several workers[39,40] have encountered numerous phenotypes in mixed cultures, but they have not studied the segregation of the supposed genic recombinants. Results obtained in the author's laboratory clearly indicated that segregants were sporadically formed during several subcultures, and that parental types were the only stable phenotypes (Table 1). Since some of these segregants transitorily exhibited a recessive phenotype of each parent, nuclear imbalance within heterokaryons alone cannot satisfactorily account for the experimental results. Continuous segregation of diverse types may be expected if the nuclei in the heterokaryon undergo several rounds of recombination; parental nuclei, however, seem to be preferentially selected.

Progress Along Other Lines

Strains of streptomycetes have been developed that are resistant to many actinophages, some of which have caused considerable loss in streptomycin fermentation. The use of resistant organisms negates the need for expensive stringent control of actinophages. Likewise, methods are being devloped for continuous-flow, open fermentation. The first step was selection of a variant that grew in liquid medium

TABLE 1

SEGREGATION FROM *Streptomyces cœlicolor* PROTOTROPHS OBTAINED BY MIXED GROWTH OF COMPLEMENTARY AUXOTROPHS

auMHSs × AUmhSr[1]

↓

(GROW TOGETHER ON COMPLETE MEDIUM)

↓

(REPLICA PLATE TO MU[2])

↓

(SPORES FROM INDIVIDUAL COLONIES ARE PLATED ONTO DIFFERENTIAL MEDIA)

↓

Subculture[3]		Derived from	No. Strains	Differential Media[4]							Phenotype
				Minimal	Complete	Streptomycin	MHA	MHU	AUM	AUH	
original		replica plate	30	+	+	−	+	+	+	+	AUMHSs
first		original	30	+	+	−	+	+	+	+	AUMHSs
second		1	30	+	+	±	+	+	+	+	AUMHSs AUMHSr
third	a	2	13	−	+	+	+	+	+	+	anomalous Sr
	b	2	9	−	+	+	+	+	−	−	AUmhSr[5]
	c	2	8	−	+	−	−	−	+	+	auMHSs
fourth	a	3a	10	−	+	+	+	+	−	−	AUmhSr
	b	3a	2	+	+	+	+	+	+	+	AUMHSr
	c	3a	1	−	+	+	+	+	+	+	anomalous Sr
fifth	a	4b	1	−	+	+	+	+	−	−	AUmhSr
	b	4c	1	−	+	−	−	−	+	+	auMHSs
	c	4b	1	−	+	−	+	+	−	−	AUmhSs
sixth		5c	1	−	+	+	+	+	−	−	AUmhSr

[1] A/a, U/u, M/m, or H/h = independent/dependent for exogenous arginine, uracil, methionine, or histidine respectively.
Ss/Sr = sensitive/resistant to 25 mg/L dihydrostreptomycin sulfate.

[2] MU, MHA, MHU, AUM, or AUH = minimal medium supplemented with methionine-uracil, methionine-histidine-arginine, methionine-histidine-uracil, arginine-uracil-methionine, or arginine-uracil-histidine respectively.

[3] Subculture inoculum consisted of spores from a single colony which was grown on complete medium.

[4] + growth equivalent to complete medium
± growth 5–20% of complete control
− no growth

[5] Parental types were confirmed by one further subculture.

as a turbid suspension. Finally, variants are being selected that are resistant to antimicrobial agents, such as propionic acid and cadmium chloride. These inhibitors can be added to unsterilized substrate before inoculation with the appropriate resistant strain, therefore making it unnecessary to sterilize the substrate or to maintain aseptic conditions during aeration, sampling, and supplementation.

Speculation

Mutants producing more active antibiotics can be screened by plating a few viable spores, which have been treated with a mutagen, on a confluent indicator bacterium of desired susceptibility. Colonies that show large zones of inhibition may be further tested for commercial application. Active, nontoxic antibiotics may be produced by variants of organisms which yield highly active but toxic antibiotics. Among recombinants, strains can be sought which produce simultaneously two or more clinically useful antibiotics; moreover recombinants from two nonproducing strains may yield commercially exploitable antibiotics.

The immediate practical applications must not obscure the equally important fundamental aspects of this investigation. The streptomycetes provide a unique opportunity to study comparatively: a) synergism, b) heterokaryosis, c) sexuality and parasexuality, and d) transduction and transformation. Moreover, the role of nucleo-cytoplasmic interactions in the *integrated cell* is becoming more apparent as these organisms are studied.[41]

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Medical School Activities

Faculty News

DR. WESLEY W. SPINK, University of Minnesota Professor of Medicine, has been appointed consultant in Internal Medicine to the Surgeon General of the United States Air Force. Dr. Spink will give the Stafford Lecture at the annual meeting of consultants January 23, 1959, at Maxwell Air Force Base, Alabama.

DR. C. WALTON LILLEHEI, Professor of Surgery in the University of Minnesota Medical School, was made an honorary member of the International Medical Club and received an honorarium for his accomplishments in the field of cardiovascular surgery.

Dr. Lillehei accepted the honor at a luncheon in Washington, D.C., where he also addressed the annual meeting of the American Association for the Advancement of Science on Tuesday, December 30.

Four times a year the International Medical Club of Washington recognizes medical men who "have reached unusual achievements in the field of medicine throughout the world."

Alumni News

DR. CARL P. MALMQUIST, M.D., 1958, University of Minnesota Medical School, submitted a manuscript entitled "Use of Tranquilizer Therapy in Office Practice" for the 1958 Schering Award competition. He was awarded an honorable mention.

IN MEMORIAM

DR. ALBERT CHESLEY, '07
Minneapolis, Minnesota

DR. DON F. FITZGERALD, '39
Minneapolis, Minnesota

DR. FRANK R. HIRSHFIELD, '20
Minneapolis, Minnesota

DR. WALTER F. MUIR, '39
Browns Valley, Minnesota

DR. FRANK J. OWENS, '55
Minneapolis, Minnesota

Coming Events

January 15	SPECIAL LECTURE: *The Viral Etiology of Spontaneous Mouse Leukemia and Its Possible Implications for the Problem of Cancer;* DR. LUDWIK GROSS, Chief, Cancer Research Unit, Veterans Administration Hospital, Bronx, New York; 125 Mayo Memorial; 2:30 P.M.
January 15–17 . . .	Continuation Course in Newer Drugs in General Practice
January 22–24 . . .	Continuation Course in Surgery for Surgeons
February 19	SPECIAL LECTURE: *Virus-Induced Acquisition of Metabolic Function;* DR. SEYMOUR S. COHEN, Charles Hayden American Cancer Society Professor of Biochemistry, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania; 125 Mayo Memorial; 2:30 P.M.
March 2–4 . . .	Continuation Course in Pediatrics for General Physicians
March 14	Continuation Course in Trauma for General Physicians
March 16–18 . . .	Continuation Course in Internal Medicine for Internists
March 30 – April 3 .	Continuation Course in Basic Concepts of Water and Electrolyte Balance for General Physicians

WEEKLY CONFERENCES OF GENERAL INTEREST

Physicians Welcome

Monday,	9:00 to 10:50 A.M.	OBSTETRICS AND GYNECOLOGY Old Nursery, Station 57 University Hospitals
	12:30 to 1:30 P.M.	PHYSIOLOGY- PHYSIOLOGICAL CHEMISTRY 214 Millard Hall
	4:00 to 6:00 P.M.	ANESTHESIOLOGY Classroom 100 Mayo Memorial
Tuesday,	12:30 to 1:20 P.M.	PATHOLOGY 104 Jackson Hall
Thursday,	11:30 A.M. to 12:30 P.M.	TUMOR Todd Amphitheater University Hospitals
Friday,	7:45 to 9:00 A.M.	PEDIATRICS McQuarrie Pediatric Library, 1450 Mayo Memorial
	8:00 to 10:00 A.M.	NEUROLOGY Station 50, University Hospitals
	9:00 to 10:00 A.M.	MEDICINE Todd Amphitheater, University Hospitals
	1:30 to 2:30 P.M.	DERMATOLOGY Eustis Amphitheater University Hospitals
Saturday,	7:45 to 9:00 A.M.	ORTHOPEDICS Powell Hall Amphitheater
	9:15 to 11:30 A.M.	SURGERY Todd Amphitheater, University Hospitals

For detailed information concerning all conferences, seminars, and ward rounds at University Hospitals, Ancker Hospital, Minneapolis General Hospitals, and the Minneapolis Veterans Administration Hospital, write to the Editor of the BULLETIN, 1342 Mayo Memorial, University of Minnesota, Minneapolis 14, Minnesota.