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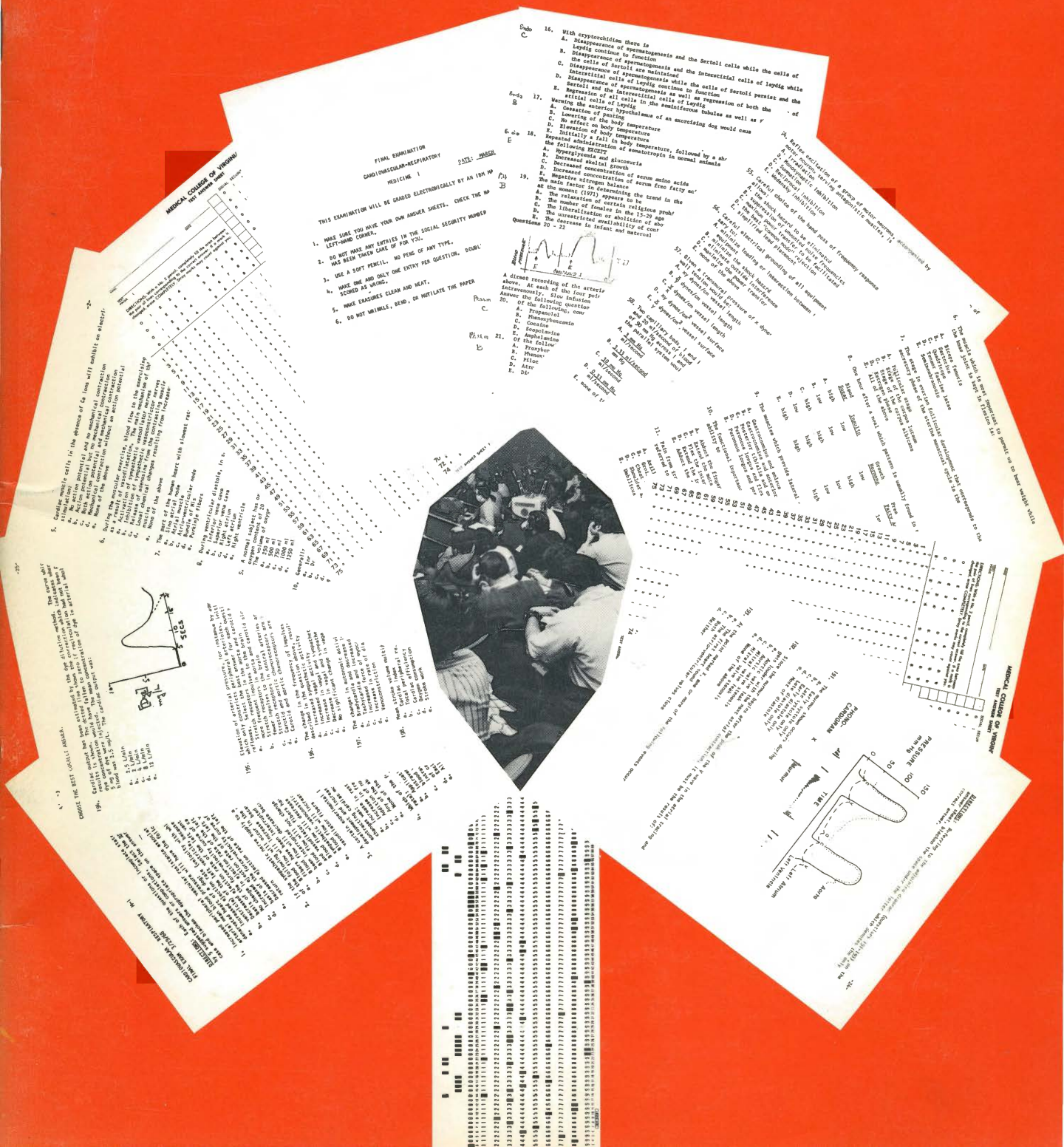

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Collusion in Multiple Choice Examinations

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Many medical schools have now adopted multiple choice examinations and computer grading. The computer may also be used to detect or confirm collusion between students in these examinations. In the following, a method is given for calculating the probability that two given answer sheets by chance agree to the extent observed.

Method

Consider a multiple choice examination. It is not necessary to assume that the number of alternatives in each question is constant. For a given pair of students,

let z = the number of questions which both students got wrong,
let x = the number of these z questions on which both students agreed,
and y = the number of these z questions on which the students disagreed.
Note x + y = z

The computer is programmed to get (x/z)%, the % agreement between the two suspected students among those questions jointly wrong; and (X/Z)%, the maximum % agreement between each of these students and every other member of the class. We can now tabulate these figures as follows:-

	No. of these with same (wrong) answer	No. of these with different (wrong) answer	Total No. of questions jointly wrong
Suspected Pair	x	y	z
Next Highest pair involving one of the above	X	Y	Z
Total	x + X	y + Y	z + Z

$$\chi^2 = \frac{(xY - Xy)^2(z + Z)}{(x + X)(y + Y).z.Z}$$

is then evaluated as a single tailed χ^2 with 1 d.f. to get P, the probability that these erroneous agreements occurred by chance.

Example

In an examination consisting of 200 multiple choice questions, two students, S_1 and S_2, are suspected of being in collusion. S_1 scored 127 (63.5%) out of 200 whereas S_2 had 105 (52.5%). Sixty-five questions were jointly wrong in these two papers and of these S_1 and S_2 agreed in 51, ie, 78%. A listing of all possible pairs including either S_1 or S_2 showed that the next highest percentage agreement among jointly wrong questions occurred with S_1 and S_{35} for which there were 53 questions jointly wrong and 26 of these agreed. The fourfold table is then:

	No. Agreeing	No. disagreeing	Total No. jointly wrong
S_1 vs S_2	51 (78%)	14	65
S_1 vs S_{35} next highest	26 (49%)	27	53
Totals	77	41	118

$$\chi^2 = \frac{(51 \times 27 - 26 \times 14)^2.118}{77 \times 41 \times 65 \times 53} = 11.13$$

$$P(\chi_1^2 \geq 11.13) < .001$$

On this basis there is less than one chance in 1000 that the two students, S_1 and S_2, accidentally agreed with each other to the extent observed or greater, ie, 51 out of 65 or 78%.

Discussion

This approach may be criticized on a number of statistical grounds but the method advocated is simple, makes few assumptions, and gives the suspected students the benefit of the doubt. The resultant probability is the chance that the two students fortuitously agree with respect to their wrong answers to the extent observed or greater as compared against the next closest pair. This of course assumes that only two students are in collusion. If there are more than two students suspected, the above test needs to be modified in an obvious way.

Alternative Methods of Grading One or More Multiple Choice Examinations

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The Single Multiple Choice Examination

The labor of grading large classes has been greatly reduced by the use of a computer equipped with an optical scanner. In examinations since 1965, students studying medicine at the Medical College of Virginia have been given precoded answer sheets containing the student's name and Social Security number. Answers to multiple choice questions are recorded by marking one of the five 'boxes' against each question number. These answer sheets are then compared automatically against a master sheet and each student's score totaled. Results for the class are printed in alphabetical and rank order giving the following information:

The number of correct answers,
The percentage of correct answers,
The "z" score, ie, (number correct − class mean)/ class standard deviation,
The standardized score, i.e. the z score standardized to a mean of 50 and a standard deviation of 10.

Students may be graded Honors, Pass, or Fail as the standardized score is greater than 70, between 70 and 30, or below (Rosinski and Hamilton, 1966).

Example: Consider a hypothetical student Y (Table 1) who scored 66 correct out of 115 multiple choice questions, each with 4 alternatives. Assume that the class mean was 79 correct and the standard deviation of the class scores was 7.3. Then Y's performance in the examination would be summarized as:

Number of questions correct = 66
Percent correct = 66/115 = 57%

$$\text{z score} = \frac{66 - 79}{7.3} = -1.78$$

Standardized score = 50 + 10(−1.78) = 32

In this section we present an alternative approach to grading in which each student's performance is evaluated without reference to his peers. Since a student's knowledge of the material may be directly estimated from a multiple choice examination, a failing grade would be given to those whose knowledge was insufficient. This would require the instructors of the course to define, before the exam, the minimum performance they would accept as satisfactory. It is felt that this would be preferable to the current practices in which either a standardized score of 30 is taken arbitrarily as the cut-off level, or the pass level is set after examining the distribution of the class's standardized scores.

Review of the Literature

In an examination of n multiple choice questions each with a alternatives, McCall (1920) relates s, the number of questions which a student might be expected to answer correctly to k, his knowledge of the material. His argument leads to the relationship:

$$s = nk + n(1 - k)/a. \qquad (1)$$

Conversely the student's knowledge of the material may be estimated by:

$$k = \left(\frac{s}{n} - \frac{1}{a}\right) \Big/ \left(1 - \frac{1}{a}\right). \qquad (2)$$

Lyerly (1951) shows that (1) and (2) are unbiased estimators of the student's "true score" and his "true knowledge" of the material.

Grading a Single Examination

In this section we consider only the classification of scores into Pass and Fail. The examiners *first* must set k_0, the minimum level of *knowledge* of the material which would be acceptable to them. This level will reflect the difficulty of the examination but a minimum of 50% knowledge is suggested as a guideline. Equation (1) then gives the number of *correct* answers in this examination equivalent to k_0. This calculation may easily be extended to allow for questions with a different number of alternatives

(or even with different values of k_0 in different sections of the exam).

It is recommended that a failing grade be given only to those students who score significantly below what can reasonably be expected from a person with a minimum passing knowledge of the material. We may, therefore, define a failing grade as a percentage score (s/n)% where

$$\frac{s}{n} < \frac{s_0}{n} - 2\sqrt{\frac{s_0}{n}\left(1 - \frac{s_0}{n}\right)\Big/ n} \qquad (3)$$

This criterion is derived from a simple χ^2 test of significance with one degree of freedom. The Type I error associated with (3) is approximately 2%.

Example: Assume that for the hypothetical examination described by Table 1, a minimum passing knowledge of 50% was set. By (1) this is equivalent to a score of 62.5% correct or 72 questions correct. The cut-off is then calculated from (3) as

$$\frac{s}{n} < .625 - 2\sqrt{.625 \times .375/115}$$

or $$\frac{s}{n} < 53.47\%$$

This is equivalent to 61.5 questions correct. Y is therefore judged to have passed the examination since his score of 66 (57%) correct is greater than 61.5 (53%), the pass-fail cut-off.

Table 1 contrasts Y's performance with the class mean, X (the minimum expected level of performance disregarding sampling variation), Z (a cut-off based on two standard deviations below the mean of the z scores), and the pass-fail cut-off defined as a level of knowledge significantly below the minimum expected. Note that this pass-fail criterion is equivalent in this example to nearly two and one-half standard deviations below the mean and could be attained by a student knowing only 38% of the material. While the definition of a failure in a single examination as illustrated may appear permissive, this depends on the choice of the minimum level of knowledge k_0. However, the application of this criterion to a student's performance over the year is as we shall see more stringent.

Promotion

Use of an Index in Promotion

Rosinski and Hamilton (1966) combine the standardized scores from a series of multiple choice questions into a Cumulative Weighted Standardized Score (CWSS). In this section we compare a number of indices including the CWSS and examine the validity of using a single figure to represent a student's performance over the year.

An overall score for the year may be defined in terms of the type of score used, its weight, and how these are combined into a single index. Many economic, demographic, psychological, and other indices are defined as linear weighted functions. This practice is also consistent (Kilpatrick, 1962) with the concept of the index as an estimator of a constant unknown parameter. In the following we consider only simple linear combinations of scores. Three types of weights are considered. *Equal weights* result in the index being the mean of the scores. The CWSS in practice uses weights proportional to the relative number of *teaching hours* in each subject. These two sets of weights will be compared against "ideal" weights generated by *principal component* analysis. In principal component analysis the class scores from a series of examinations are restructured as orthogonal (uncorrelated) linear combinations of the original scores. Not only are these

TABLE 1

Equivalent scores in an examination of 115 multiple choice questions, each with four alternatives.

	Fail level	Z	Y	X	Class Mean
Number correct	61.5	64	66	72	79
% correct	53.5	56	57	62.5	69
Number known	44	47	50	57.5	67
% known	38	41	43	50	58
z score	−2.40	−2.00	−1.78	−0.68	0.00
Standardized Score	26	30	32	43	50

X—scores equivalent to 50% knowledge
Y—a hypothetical student's score
Z—scores equivalent to two standard deviations below the class mean
Fail level—scores equivalent to a level of knowledge significantly ($P < 0.05$) below the desired minimum of 50% knowledge

TABLE 2

For a given class of medical students, the table gives the percentage of variation in the number of correct questions extracted by an index of the type shown.

Score Used in Index	Weights Used in Index		
	Principal Component	Equal	Teaching Hours
Number correct	49.96	49.76	48.69
Estimated % Knowledge	49.88	49.78	49.17
Standardized Score	49.78	49.49	49.93*
Rank	49.12	48.86	48.22

* Cumulative Weighted Standardized Score as used at the Medical College of Virginia. This includes bonus questions and other artifacts which in this particular case result in an index extracting more of the variation than is theoretically possible.

components independent, but the weights are adjusted so that the first or principal component extracts or explains the maximum degree of variability that can be extracted by any linear index. The second and remaining components are defined in decreasing order of the amount of variability extracted (Harman, 1967).

Each of these three weighting systems is used in the formation of an index combining the four possible scores available. These are: *the number of correct questions;* the standing or *rank of a student* in the class based on the number of questions answered correctly; the *standardized score* defined in the introduction; and the estimated *knowledge* level defined in equation (2), or the percent of questions the student is estimated to know without guessing.

These 12 indices (three weighting systems by four types of scores) were calculated for each of the 86 students who completed the second phase of the integrated medical curriculum at the Medical College of Virginia. (Table 3 lists the 14 component examinations in this phase.)

The relative amount of variability extracted by each index is shown in Table 2. Surprisingly each of the 12 indices leaves more than 50% of the variability among students' scores unaccounted for. In other words, if this data is typical, a single linear combination of scores from different examinations will describe no more than about half of the differences among students' scores over the 14 examinations. Since the percent variability extracted by these indices are approximately equal, they are all equally *uninformative.*

We now investigate whether by using two indices we can increase the percent variation accounted for. This would be equivalent to representing a student's overall performance as a point on a graph rather than as a point on a line. The second principal component which by definition extracts the maximum of the remaining variability added only 8.6%. Thus, using two figures to represent 14 examination results accounts for, at most, only 58.6% of the total variability in the data used here. As one or even two indices cannot adequately represent a student's performance over a year, we now consider the application of the criterion introduced in the first section to the problem of promotion.

Unsatisfactory Performance in a Series of Examinations

In the first section it is recommended that a failing grade be given to students who score significantly less than that expected of a person with a minimum acceptable knowledge of the material. We recommend the same criterion for promotion, viz. that a student would not be promoted if his total score for the year or phase was significantly less than that expected of a hypothetical student who, in each component examination, knew only the minimum acceptable.

The cut-off for the year would be calculated as before using equation (3) except that now, s_0 would be defined as the sum of the s_0 scores in each examination and n would be the total number of questions given in all examinations.

Example: Table 3 shows the application of the criterion to each of 14 examinations in the second phase of the medical curriculum. The sum over all 14 examinations of s_0 is the number of correct questions which might be expected of a student with a minimum acceptable knowledge throughout the phase. This is equivalent to 67% correct or 56% knowledge of the material examined. Following the same procedure as before (shown on last line of Table 3) and using n = 1821, the total number of questions, we find the cut-off for the year to be 65%, or more accurately, 1180 questions correct out of 1821.

Note that this cut-off level is only 2% below the level expected from a person with minimum acceptable knowledge. This is in contrast to the difference between the cut-off level and expected minimum in a single examination. Thus, in Table 1, the cut-off level (53.5%) is 9% below the desired minimum of 62.5%. With nearly 2000 questions we can detect more readily those students whose knowledge falls significantly below the acceptable minimum for the year.

Table 3 shows that in typical examinations the minimum passing score in terms of percent correct is generally greater than the minimum passing level of knowledge. The requirement that a student needs to score more than 65% correct over the year is here based on minimum pass levels in the separate exams ranging from 48% to 72% correct. These in turn derive from minimum acceptable levels of knowledge ranging from 40% to 70%. For the benefit of readers used to standardized scores, Table 3 gives the standardized scores equivalent to the hypothetical passing levels adopted here.

In Table 4, Y's performance is compared with these cut-off levels in each of the 14 component examinations and overall. It is seen that Y knew significantly less than the minimum in the reticulo-endothelial, cardiovascular, endocrine, gastrointestinal, and written comprehensive sections of the year. Even if he had not failed these sections, Y should not be promoted since over the whole year he scored less than the minimum of 65% correct. Therefore, Y's knowledge of the material in this phase of the curriculum fell significantly below the (hypothetical) minimum level of 56% knowledge required of students for promotion.

Discussion

Use of the standardized score from a single examination to award grades of Pass or Fail implies that students are judged against their peers and that no absolute standard is possible. The alternative proposed here is to use the percent of questions *known* for evaluation. To estimate the percent of questions known, it is assumed that when a student does not know the answer he guesses among the alternatives. It is further assumed that in such guessing each alternative is equally likely. Clearly these assumptions are only a first approximation. However, this ap-

TABLE 3

Showing the calculation of the pass-fail criterion based on hypothetical minimum acceptable levels of knowledge k_0.

Examination	n	k_0 %	(s_0/n) %	s_0	$(1-s_0/n)$	$s_1 = s_0(1-s_0/n)$	$\sqrt{s_1}$	Pass-Fail Cutoff: Score $s_f = s_0 - 2\sqrt{s_1}$	Pass-Fail Cutoff: Percent Score $(s_f/n)\%$	Pass-Fail Cutoff: Equivalent Standardized Score
Reticulo-endothelial	112	50.	62.5	70.0	.375	26.25	5.1	59.8	53.4	25.3
Infectious Diseases	115	50.	62.5	71.9	.375	26.96	5.2	61.5	53.5	25.7
Pharmacological Agents	98	50.	62.5	61.3	.375	22.99	4.8	51.7	52.8	6.0
Pathogenesis	113	55.	66.3	74.9	.337	25.24	5.0	64.9	57.4	14.1
Cardiovascular Respiratory	214	70.	77.5	165.9	.225	37.33	6.1	153.7	71.8	41.4
Urinary	99	70.	77.5	76.7	.225	17.26	4.2	68.3	69.0	34.9
Endocrine	118	70.	77.5	91.4	.225	20.56	4.5	82.4	69.8	31.3
Gastrointestinal	147	45.	58.8	86.4	.412	35.60	6.0	74.4	50.6	37.2
Man in his environment	110	60.	70.0	77.0	.300	23.10	4.8	67.4	61.3	41.1
Musculo-skeletal	112	60.	70.0	78.4	.300	23.52	4.8	68.8	61.4	32.6
Clinical Medicine	100	70.	77.5	77.5	.225	17.44	4.2	69.1	69.1	23.1
Central Nervous System	177	40.	55.0	97.4	.450	43.83	6.6	84.2	47.6	38.6
Comprehensive Practical	66	50.	62.5	41.3	.375	15.49	3.9	33.5	50.8	43.1
Comprehensive Written	240	50.	62.5	150.0	.375	56.25	7.5	135.0	56.3	42.4
Second Phase	1,821 (sum)	56.	67.0	1220.1 (sum)	.330	402.63	20.1	1179.9	64.8	41.9

TABLE 4

An example of Y's performance in the second phase of an integrated medical curriculum in which the examinations consist of multiple choice questions, each with four alternatives.*

Examination	n	Pass/Fail % Correct	Summary of Y's Performance			
			% Correct	% Known	Standardized Score	Grade
Reticulo-endothelial	112	53	52	36	29	F
Infectious Diseases	115	54	57	43	32	
Pharmacological Agents	98	53	67	56	36	
Pathogenesis	113	57	65	53	30	
Cardiovascular Respiratory	214	72	67	56	40	F
Urinary	99	69	76	68	47	
Endocrine	118	70	62	49	29	F
Gastrointestinal	147	51	48	30	36	F
Man in his environment	110	61	67	56	56	
Musculo-skeletal	112	61	71	61	49	
Clinical Medicine	100	69	82	76	45	
Central Nervous System	177	48	64	52	44	
Comprehensive Practical	66	51	64	52	59	
Comprehensive Written	240	56	50	33	32	F
Final Evaluation	1,821	65	62	50	36	F

* Scores are hypothetical.

proach is useful in defining the minimum number of questions which will be considered satisfactory in a given examination. If, in a multiple choice examination of 100 questions with four alternatives per question, 63 correct is set as the minimum passing level, this score can be made by a student who knows and answers 50 questions correctly and who then selects his answers to the remaining 50 at random. A student who knows the answers to 50 of the questions and who has partial knowledge of the remaining 50 questions should be able to score higher than 63 because he will be able to exclude some of the alternatives in each question before guessing the answer. Likewise, a student who knows the answers to 26 questions but is able to exclude two of the four alternatives in each of the remaining 74 will on average also answer 63 questions correctly. Use of the estimated percent of questions known is recommended, therefore, not as a model of what happens in a multiple choice examination but as a means of providing the faculty with a procedure for establishing minimum pass levels.

The foregoing analysis, however, raises the question of the utility of a system in which the student is examined on average every month. It is impossible to discriminate in a multiple choice examination between a student who knows half of the questions and guesses the remainder, and one who has a broad but partial knowledge of the subject which enables him to exclude many of the alternatives and select the correct answer with a high degree of probability by a process of exclusion. In a series of multiple choice examinations, neither the Combined Weighted Standardized Score nor any other linear index proved to be satisfactory because students in the class chosen for analysis exhibit varying degrees of knowledge in the different examinations. Examinations are useful as a teaching device and this aspect needs to be more emphasized, especially with students. One way of achieving this is to retain the current series of examinations throughout the year but to stop grading these examinations. They could still be scored by the computer and the results generally released.

The student would not be promoted if his 'average' over the year fell below the cut-off for promotion. This knowledge would be a sufficient stimulus to motivate the student to learn the material presented in a given year or phase of the medical curriculum. The idea is similar to the requirement that college students, to remain in good academic standing, maintain a B average.

In contrast, the examination procedure which has evolved with the integrated curriculum has degenerated into a series of competitions in which the last two or three students in each examination are judged

to have failed. This arises from an uncritical adherence to the use of a standardized score of 30 as a cut-off, since two standard deviations below the mean will, in a normal distribution, exclude about two in 100 students. Moreover, the use of the percent correct and hence the standardized score is unfair. Hamilton (1950) shows that scores in multiple choice examinations are biased upwards, the student who knows fewer of the answers gaining more from this bias than those who know the material well.

At the Medical College of Virginia, the Combined Weighted Standardized Score is calculated for each medical student at the end of each phase of his training, using weights proprotional to the number of hours taught in each system. The comprehensive examinations in the second phase are arbitrarily given 25% of the total weight. The CWSS's are used to rank the students and this ranking of students is used during promotion considerations. Since the CWSS, together with other indices, leaves half of the total variability of students' performance unaccounted for, this procedure is clearly not too satisfactory. The promotions committee evaluates students against a CWSS equivalent to the minimum passing level in each section (in the event of there being no failures in a section, the lowest standardized score in that examination is used). This criterion is rather arbitrary and, although other information is considered, the decisions reached regarding promotion are subjective. The faculty are hindered rather than helped by the CWSS which has no clear interpretation. This is evidenced by the erroneous assumption that the CWSS has a standard deviation of 10. The CWSS has a standard deviation smaller than 10 because of a positive correlation among a student's scores over a year. Thus, at the end of the second phase Y would be considered for promotion using a CWSS of 41 rather than a standardized score of 36 or 1.4 standard deviations below the class mean (Table 4).

Summary and Conclusion

This paper describes the use of the standardized score in grading multiple choice examinations by computer. Since standardized scores make no allowance for guessing, it is recommended that the percent of questions *known* be used instead, and that a student who correctly answers significantly fewer than a predetermined number of questions be given a failing grade.

The performance of a class of sophomore medical students in a series of 14 multiple choice examinations was analyzed. It was found that no single linear combination of their scores could account for more than 50% of the variability among students over the year. Therefore, it is recommended that the grading of subject matter examinations into Pass or Fail be ended. Promotion would not, however, be granted to those students who scored significantly below that expected of a person with overall minimum knowledge of the material. Students entering this system would be informed of the overall minimum passing level for that year or phase and given, after each section examination, their cumulated score of questions correctly answered. It is considered that a change such as this toward liberalization of the examination system would be welcomed by students and faculty alike.

References

Hamilton CH: Bias and error in multiple choice tests. Psychometrika 15: 151, 1950

Harman HI: Modern Factor Analysis. Chicago, University of Chicago Press, 1967

Kilpatrick SJ: Occupational mortality indices. Population Studies 16: 175, 1962

Lyerly SB: A note on correcting for chance success in objective tests. Psychometrika 16: 21, 1951

McCall WA: A new kind of school examination. Educational Research 1: 33, 1920

Rosinski EF, Hamilton DL: Examination procedures as part of a new curriculum. J Med Educ 41: 135, 1966

The Use of Principal Component Analysis to Increase the Ability of Multiple Choice Examinations to Distinguish Among Students*

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Introduction

Multiple choice examinations are widely used in standard examinations such as the GRE, MCAT, or Medical Board examinations. This type of examination has also gained considerable popularity as a means of examining students on the material presented in a formal course of instruction, especially when classes are large. This popularity is due at least in part to the ease with which such examinations can be graded. Indeed, it is often possible through the use of standard answer forms to have the examination graded entirely by computer (Rosinski and Hamilton, 1966).

The widespread use of the multiple choice examination has led to much discussion of its ability to evaluate examinees' knowledge of the material on which they are examined. The effectiveness of the evaluation depends, of course, on the objectives of the examiner. Various practical and/or philosophical reasons have been given for such evaluations (Karsner, 1937). We shall not discuss these but shall assume that one purpose of administering an examination is to distinguish among examinees with respect to knowledge of a specific subject area. Attention is focused here on the examination's ability to achieve this purpose; in particular, a method of scoring is presented which provides a greater distinction among examinees than the usual methods of scoring.

An objection often voiced against multiple choice examinations is that questions are sometimes stated ambiguously so that two or more choices appear to be equally correct. Neither these questions nor those for which either all or none of the examinees know the correct answer contribute to the distinction among examinees. Indeed, if all questions were of these types, the only variation among test scores would be chance variation due to guessing. On the other hand, questions for which some but not all individuals know the correct answer reflect variation among individuals' knowledge of the material covered on the examination as well as some chance variation due to guessing. Thus, it would seem desirable to eliminate all questions which contribute only chance variation to the test scores. The result would be a set of test scores with a higher variance which is influenced less by chance variation due to guessing.

Pratt and Ingersoll (1968) present a method which attempts to detect questions which contribute little to the distinction among examinees' knowledge. These questions are then eliminated and test scores based only on the remaining good questions are obtained. Their procedure is based primarily on intuition and uses a quite arbitrary criterion for determining "good" questions. However, for the two tests to which they applied the method, they were successful in obtaining test scores based only on "good" questions which had a considerably larger variance than the scores based on all questions.

In this paper we present a method of scoring, based on the statistical technique of principal components, which yields a set of test scores having maximum variance. In addition, it is demonstrated that this method can indicate questions which contribute little or nothing to the distinction among examinees. This procedure is applied to two tests, and

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the scores obtained are compared with the usual percentage scores. The scores are also compared to those obtained by the method of Pratt and Ingersoll for one of the tests.

Methods

In the description of methods, we shall have need to refer to an individual's score on a single question or item as well as to his score on the entire test. In order that the distinction be clear, the score on the entire test will be referred to as the test score and denoted by T, while the score for a specific item will be referred to as an item score and denoted by S. For the usual methods of scoring, the test score can be written as a linear combination or "weighted" sum of the individual's item scores. Thus

$$T = \sum wS \qquad (1)$$

where w represents the weight assigned to a specific item. For example, a common method of scoring is to assign as the test score for an individual, the percentage of test questions answered correctly. This can be obtained from the above formula by assigning an item score of 1 or 0 depending on whether the answer given is correct or incorrect and by assigning a weight of $w = 100/n$ to each question, where n is the number of questions on the examination.

In order that test scores be comparable, they should be based on the same method of assigning item scores. We shall adopt the convention of assigning an item score of 1 for a correct answer and an item score of 0 for an incorrect answer. The only difference among tests scores obtained by different methods will then be determined by the weights assigned to the specific items.

Since we shall apply the method of Pratt and Ingersoll, as well as our own, a description of their method is in order. They first divide the examinees into an upper and lower half based on the percentage of correct responses for the entire test. An item is then declared to be a "good" question if the percentage of upper half students giving the correct answer is at least eight percentage points higher than the percentage of lower half students answering the question correctly. The test score assigned for an individual is then the percentage of "good" questions answered correctly. This score can be obtained from (1) by assigning all "good" questions a weight of $w = 100/n_g$, where n_g is the number of good questions, and a weight of $w = 0$ to all other questions.

Note that the method of Pratt and Ingersoll constitutes a differential weighting of item scores. This is done in an attempt to obtain a set of test scores with greater variance than the usual percentage scores, thereby making the distinction among students clearer. However, some "good" questions are certainly better than others for purposes of distinguishing among students. Also, some "good" questions are only slightly better than those not considered to be "good" questions. It would seem therefore that a more judicious assignment of differential item weights might yield test scores with an even larger variance. In fact, one might assign item weights in such a way that the variance among test scores is a maximum for all methods of scoring of the type $T = \Sigma\, ws$. Such a set of weights can be obtained from a principal components analysis of the item scores. The weights used are those corresponding to the first principal component of the item scores. A description of principal components analysis can be found in most texts on multivariate analysis (Morrison, 1967; Seal, 1962). Standard computer programs for performing such an analysis are also available at most scientific computing installations.

In assigning item weights, the relationship between item scores for different items should be taken into consideration as well as the variance of item scores for a specific item. For example, if the covariance (and hence the correlation) between two items is large, both items are likely to be measuring the same aspect of knowledge. Consequently, both questions, even though they may be good at distinguishing among individuals, should not be given large weights relative to other questions. The method of principal components takes care of this situation by either assigning a larger weight to the better of the two questions or by assigning intermediate weights to each of the two questions. Thus, the actual value of a weight cannot be taken as a complete indication of a question's utility in distinguishing among students. In general, however, a very small weight will indicate a question that is of little value in distinguishing among examinees.

In the present application, it is desirable that all weights be positive to insure that no examinee be penalized for correctly answering a question. In general, not all weights obtained through the method of principal components are positive. However, a theorem due to Perron (1970) asserts that if all covariances (and thus correlations) between items are positive the weights associated with the first principal component will also be positive. Certainly all correlations between test items should be positive, for a negative correlation would indicate that a correct response to one question tends to be associated with an incorrect response to another. This should only be the case if an incorrect choice is indicated as the correct answer for one of the questions. However, we are dealing with estimates of the true covariance between items, and it is possible to obtain a negative value as an estimate even though the true covariance is positive. This will generally occur only if the true covariance is near zero, which usually

results from one of the items being such that very few if any of the examinees know the correct answer. Thus, negative covariances, and hence negative weights, indicate questions that are of little value in distinguishing among examinees. These questions are therefore assigned a weight of zero. However, since negative weights may be indicative of questions for which an incorrect choice is designated as the correct answer, a closer scrutiny of these questions may be warranted.

The method of scoring based on a principal components analysis of item scores was applied to two tests. The first test was an examination given to 127 first year medical students at the Medical College of Virginia during the 1968–1969 school term. The examination, consisting of 45 multiple choice questions on statistics and mathematics, was given prior to a course in biostatistics. The purpose of the examination was to divide the class into an advanced and elementary section if the students' backgrounds varied greatly. Thus, the examination contained several questions for which few students knew the correct answers. The second test, consisting of 50 multiple choice questions, was the final examination in biostatistics given to 134 first year medical students during the 1969–1970 school term. For purposes of comparison, the method of Pratt and Ingersoll was also applied to the first test.

Results and Discussion

As noted previously, the variation among test scores is a useful index of the scores' utility in distinguishing among examinees. A measure of this variation is provided by the standard deviation of the test scores. For the methods of scoring applied to the first test described above, the standard deviations were 8.8 for the method of percentage scores, 12.8 for the method of Pratt and Ingersoll, and 37.0 for the method based on a principal components analysis of item scores. While the method of Pratt and Ingersoll offers some improvement over the method of percentage scores (the improvement observed here is consistent with that reported in Pratt and Ingersoll, 1968) the method based on principal components offers a substantial improvement over either of the other methods.

The percentage scores and the corresponding principal components scores for the 15 students with the highest and lowest percentage of correct answers on the first test are presented in the Table. In addition to the greater variation among the scores based on principal components, it may be observed that the principal components score is higher than the corresponding percentage score for all except two of the top 15 students. Similarly, the principal components score is lower than the corresponding percentage score for all but one of the bottom 15 students. In

TABLE

Percentage scores and principal components scores for the first and last fifteen students on the basis of their percentage scores on test 1. (A total of 127 students took the examination.)

Top 15 Students		Bottom 15 Students	
Percentage scores	Principal components scores	Percentage scores	Principal components scores
80.0	91.9	44.4	42.7
77.8	87.8	42.2	33.4
73.3	79.6	42.2	41.5
73.3	75.4	42.2	40.8
71.1	71.2	42.2	31.7
68.9	77.3	40.0	24.2
68.9	80.0	40.0	26.7
68.9	67.9	40.0	29.8
68.9	83.3	37.8	34.6
66.7	83.9	37.8	33.5
66.7	79.1	37.8	36.9
66.7	80.6	37.8	20.6
66.7	84.8	37.8	27.7
66.7	64.2	35.6	18.8
66.7	74.4	33.3	36.7
Mean 65.3	78.8	39.4	32.0
Range 13.3	27.7	11.1	23.9

general, therefore, a student near the top (bottom) of the class with respect to the percentage scores will also be near the top (bottom) with respect to the principal components scores. It should be noted, however, that within the top 15 students, the students' ranks based on the two methods of scoring differ considerably, and similarly for the bottom students. This is to be expected since some students are more fortunate than others in guessing the correct answer to non-discriminating questions such as those for which no students know the correct answer.

It may also be observed from the Table that six of the top 15 students received identical percentage scores of 66.7. On the other hand, the principal components scores for these students ranged from 64.2 to 84.8. Thus, where no distinction was possible on the basis of their percentage scores, a fairly wide distinction is possible on the basis of their principal components scores. Further, examples of this type are available from an inspection of the data in the Table.

Application of the method of principal components to the first test considered yielded 13 negative weights. An item analysis of the question corresponding to these weights revealed that the percentage of correct responses to all except one of these questions was quite low. In fact, the percentage was about what would be expected had all students made their choices completely at random. For the other question to which a negative weight was assigned, the percentage of correct responses for the lower half of the class was 46% as compared to 52% for the upper half. Further investigation of the responses to this question indicated that the majority of the class was able to narrow the alternatives to two choices but had to guess between these two. Thus, it is apparent that these 13 questions could contribute nothing to the distinction among students. It would thus appear that it is indeed appropriate, as indicated in the methods section, to assign these questions a weight of zero for purposes of computing test scores. It should also be noted that each of these questions was declared to be a "poor" question and thus assigned a zero weight by the method of Pratt and Ingersoll as well.

Further investigation of the manner in which item weights were assigned by the method of principal components revealed that the larger weights were associated with questions for which the difference between the percentage of correct responses by the upper and lower halves of the class was greatest. For example, the largest weight was assigned to the question having the greatest difference between the percentage of correct responses (71% for the upper half versus 32% for the lower half). Similarly, lower weights were associated with questions for which the differences between the upper and lower halves of the class were small.

Application of the method of scoring based on principal components to the second test described above produced results similar to those for the first test. The standard deviations of test scores was 13.0 for the percentage scores and 38.8 for the principal components scores. The relationship between the magnitude of item weights assigned by the method of principal components and the difference between the percentage of correct responses by the upper and lower halves of the class was also similar.

On the second test, however, no negative weights were encountered. This is apparently due to the fact that there were few if any questions for which no students knew the correct answer. This is to be expected since this was a final examination on which much care was taken not to include questions on material which had not been covered in class. On the other hand, the first test was given in an effort to determine students' backgrounds prior to presenting a course of instruction and consequently contained several questions for which very few students knew the correct answer. This indicates that with careful construction of multiple choice examinations, questions with no discriminating ability can be avoided; however, even then test scores based on a principal components analysis of item scores are markedly superior to percentage scores for distinguishing among examinees.

Summary

A method of scoring multiple choice examinations based on the statistical technique of principal components analysis is described. This test is applied to two tests. The results indicate that the method yields test scores which have a marked advantage over the usual percentage scores in distinguishing among examinees with respect to knowledge of subject matter. In addition, the method provides an indication of whether a question is "good" or "poor" for purposes of distinguishing among examinees.

References

Karsner HT: Philosophical comments on examinations. JAMA 108: 1022, 1937

Morrison DF: Multivariate Statistical Methods. New York, McGraw-Hill, Inc., 1967

Perron O: Zur Theorie der Matrices. Math Ann 64: 248, 1907

Pratt PC, Ingersoll RW: A method for increasing the reliability and validity of multiple choice examinations. J Med Educ 43: 1238, 1968

Rosinski EF, Hamilton DL: Examination procedures as part of a new curriculum. J Med Educ 41: 135, 1966

Seal HL: Multivariate Statistical Analysis for Biologists. New York, John Wiley and Sons, Inc., 1962

A Note on the Probability of Misclassifying Students on the Basis of a Multiple Choice Examination

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Introduction

As college enrollments continue to increase, the amount of time spent by faculty members grading test papers is also increasing. Often this extra time is taken at the expense of research and other teaching duties. In an effort to regain this time, many educators are giving multiple choice examinations and using the computer to: obtain the number of questions answered correctly, standardize the scores, rank the students, analyze the test to determine if it actually does discriminate among the students, and obtain various other parameters which are of interest to the teacher. After obtaining this information from the computer, the decision still has to be made as to which students failed to exhibit a satisfactory amount of knowledge of the subject matter on the test. This usually results in the students being assigned to groups such as excellent, pass, fail, or A, B, C, D, F. However, since a test can only sample a student's knowledge of the subject matter, misclassifications will occur, eg, a student placed in the pass group really belongs in the fail group or vice versa.

Since testing is somewhat analogous to random sampling, statistical theories find an application in the general problem of classifying students on the basis of examination performances. Here a method of calculating the probability of misclassification of students on multiple choice examinations will be discussed and applied to a test. The probability of misclassification is the probability that, in a comparison between two students, the student who gave the correct answer to fewer questions, say Z_1, actually knew the correct answers to as many or more questions than the student who gave the correct answer to Z_2 questions ($Z_2 > Z_1$). Such an event can occur because students can guess the correct answer to a question for which they do not know the answer.

Krutchkoff (1967) has defined the separation level of grades as the probability that a student with a higher grade actually knew the answers to more questions than the student with a lower grade. This is very similar to the probability of misclassification defined above. However, to arrive at an expression for the separation level of grades, it was necessary to make two rather restrictive assumptions:

1. Partial knowledge plays no role in a student's guess at the answer to a question for which he does not know the correct response.
2. The class of students taking the examination is homogeneous.

The first restriction is clearly too restrictive, as illustrated by a hypothetical example. Consider a student who does not know the correct answer to a given question which contains four possible answers. As a result of his partial knowledge of the subject matter, this student is able to eliminate as incorrect two of the possible answers. Hence, the student is now able to guess the correct answer with probability ½ instead of ¼. The second assumption is not a realistic one and is unnecessary in the derivation of the probability of misclassification. For a mathematical derivation of the probability of misclassification, see Carter (1971).

The probability of misclassification is based on each student's partial knowledge of the subject

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TABLE 1*

	9(5, 4)	9(7, 2)‡	9(7, 2)‡
8(6, 2)†	0.839	0.889	0.332
8(3, 5)	0.410	0.478	0.047
8(4, 4)	0.448	0.516	0.063
8(5, 3)	0.410	0.478	0.047
8(7, 1)	0.477	0.545	0.077

Krutchkoff's separation level = 1 − PMC(8, 9) = 0.558
PMC(8, 9) = 0.442, where PMC is the probability of misclassification.

* The entries in Tables 1, 2, and 3 are the PMC's, ie, in the i, j position we have tabulated the probability that student i actually knew as many or more correct answers than student j.

† 8(6, 2) denotes a student who answered 8 questions correctly, 6 on one half of the test and 2 on the other half.

‡ These two students have different guessing distributions.

matter. Since we have seen how guessing can be affected by partial knowledge, it is reasonable to consider the probability of guessing correctly the answer to a question as a random variable. This is contrary to what is usually done. The probability of guessing the correct answer to a question is customarily taken to be $1/r$, where r is the number of possible answers to a question. In this paper, this probability is considered to be a random variable possibly taking on a different value for each individual taking the test. It is assumed that this variable follows a Beta destribution with unknown parameters which must be estimated.

As a result of the effect of partial knowledge on guessing, and since guessing only occurs on questions for which the answer is unknown, it seems appropriate to include on the examination several questions chosen such that the students would not be expected to know the answer. However, these questions should be chosen in such a manner as to allow a student's partial knowledge to aid in arriving at the answer. The parameters of this Beta distribution are then estimated for each student from his performance on these questions using a method due to Weiler (1965).

Application

The methods developed here were applied to an examination given by the Biometry Department to 127 first year medical students at the Medical College of Virginia in September 1968. The test was given to determine the mathematical and statistical backgrounds of these students. The students with high grades were to be assigned to a more advanced course in statistics than those with lower grades. It was decided that those students who correctly answered nine or more questions were to be placed in the advanced course and those who answered fewer than nine questions in the elementary course. Since there were five students who answered eight questions correctly and three who answered nine questions correctly, it was of interest to calculate the probability of misclassification for each pair of students with these two scores.

To estimate the parameters of the underlying Beta distribution, the examination was randomly divided into two parts such that on each part there was an approximately equal number of questions designed to measure a student's partial knowledge. The probabilities of misclassification were then calculated for the students (Table 1).

Since students will generally perform differently on the set or sets of questions designed to measure their partial knowledge of the subject matter, it is possible, by using the method just discussed, to calculate the probability of misclassification for students who have correctly answered the same number of questions. This is useful in that we can now rank

TABLE 2
The Probabilities used to Rank Students who Correctly Answered 8 Questions

	8(6, 2)	8(3, 5)	8(4, 4)	8(5, 3)	8(7, 1)
8(6, 2)		0.935	0.915	0.935	0.897
8(3, 5)			0.561	0.606	0.529
8(4, 4)				0.638	0.563
8(5, 3)					0.529

The assumptions made in the derivations of Krutchkoff's separation level will not permit the calculation of a separation level for students who answered correctly the same number of questions.

students who have the same raw score by calculating the probability that one such student knew the answer to more questions than another student with the same number of correct answers. This probability has been calculated for the students who correctly answered eight and nine questions and the results appear in Tables 2 and 3, respectively.

Conclusion

The students' ability to guess correctly questions on a multiple choice examination creates a problem in the determination of the number of questions the students actually knew. Therefore, it is not unlikely that mistakes will be made when grades are assigned. In this paper, a method for calculating the probability of misclassification of students as a result of a multiple choice test in which the assumption of a uniform guessing distribution is relaxed has been discussed and illustrated. To do this it is necessary to include on the examination several questions, related to the subject matter on which the students are being examined, for which the students will have to guess the answer.

Another problem which frequently occurs in the evaluation of students' performances is the assignment of meaningful class ranks to students who correctly answer the same number of questions. However, since students usually will perform differently on the set of "guessing" questions, it was shown that it is possible to calculate the probability that one student knew the answer to more questions than another student who correctly answered the same number of questions.

TABLE 3

The Probabilities used to Rank Students who Correctly Answered 9 Questions

	9(5, 4)	9(7, 2)	9(7, 2)
9(5, 4)		0.636	0.123
9(7, 2)			0.082

The author recognizes the need for placing confidence intervals on the estimated probabilities of misclassification. However, to calculate such quantities, a knowledge of the distribution of these probability estimates is necessary. This is not known and mathematics needed to arrive at this distribution would be extremely complicated.

References

Carter WH: The probability of misclassification of students on multiple choice examinations. The Journal of Educational and Psychological Measurement, December 1971, in press.

Krutchkoff RG: The separation level of grades on a multiple choice examination. The Journal of Experimental Education 36: 63, 1967

Weiler H: The use of incomplete Beta functions for prior distributions in binomial sampling. Technometrics 7: 335, 1965

A Survey of Laboratory Programs for First Year Medical Students

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Introduction and Methods

Basic science courses offered to freshmen medical students have been traditionally taught by didactic presentations and laboratory work. Various factors have prompted many departments to either markedly reduce the time allotted for the traditional, but more vulnerable, laboratory phase of these courses or drastically alter the content of the laboratory programs. Information concerning the desirability for such changes is incomplete and no evaluation has been developed to determine the effectiveness of change. Before beginning any further alterations in the laboratory programs at the Medical College of Virginia, it seemed desirable to determine anew what we wish to teach in the laboratory. This problem was approached in part by surveying the attitudes of other anatomy, biochemistry, and physiology departments.

Questionnaires were sent to the chairmen of anatomy, biochemistry, and physiology departments in 116 medical schools. These schools included 102 US medical schools, 12 Canadian medical schools, and 2 American type medical schools in foreign countries. The US schools included some new medical schools which have opened only recently or which are scheduled to begin classes in Medicine within the next few years. Seventy-one anatomy departments, 70 biochemistry departments, and 72 physiology departments responded by returning completed or at least partially completed questionnaires. (Incomplete answering of questionnaires by some departments resulted in slight variations from question to question in the total number of departments responding.) The data were obtained with the promise of anonymity and, although some direct quotations will be included, anonymity will be preserved throughout this report.

The first section of the questionnaire attempted to determine the characteristics and teaching responsibilities of the departments responding. The second section of the questionnaire was designed to indicate the significance which the departments place on the laboratory and to establish how they conduct their laboratory programs.

One part of the questionnaire listed seven possible objectives of a laboratory program:

1. Interpretation of clinical laboratory findings
2. Acquirement of manipulative skills
3. Supplementation and reinforcement of didactic material
4. Development of student-faculty relations
5. Appreciation of experimental development and methodology
6. Experience and responsibility of working with live animals or tissues
7. Other (to be specifically stated)

The department chairmen were then asked to rate these objectives using a system of 1 through 4 with 1 representing a very important objective and 4 representing an objective of much lesser importance. In evaluating the returns we considered a rating of 1, 2, or a checkmark to indicate an important objective and other ratings to denote relatively unimportant objectives.

The data concerning the three disciplines of anatomy, biochemistry, and physiology were analyzed separately using an IBM 1130 computer. The

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results associated with each discipline are also reported separately for the convenience of those with discipline-oriented interest.

Some Characteristics and Teaching Responsibilities of the Departments

According to the departments that responded to the questionnaire, the average anatomy department consists of 10.6 full time faculty members and 2.9 part time members. Of the total 66 replies analyzed, 63 teach graduate students in addition to medical students; 25 have responsibilities in dental, 24 in nursing, 21 in physical therapy, 10 in dental hygiene, and 6 in pharmacy curricula. There are 26 departments that have commitments in disciplines other than those listed in the questionnaire. These include occupational therapy, medical technology, veterinary medicine, postdoctoral education, residents, and undergraduate courses in the arts and sciences. Forty-nine of the 66 anatomy departments analyzed are at institutions operating under the traditional program based on the departmental courses. Fourteen departments are part of an integrated program involving at least one other department. Three departments felt their program did not fit any of the listed categories. Of 64 replies, 33 departments still identify their courses of neuroanatomy while 31 list their course as an integrated neural science course.

The departments of biochemistry responding to the questionnaires report a mean of 9.7 full time and 5.8 part time faculty. All of these departments teach medical students; most teach graduate students; almost a third teach dental students; and some teach various other students in paramedical, agricultural, or liberal arts areas. Fifty-nine of 67 departments are at medical schools which utilize separate departmental courses; the remaining eight departments teach medical students in an integrated program involving other departments.

The physiology departments responding to our questionnaire make up a population which has an average of ten full time faculty members and three part time members. Of the total 72 physiology departments responding, 70 teach graduate students in addition to medical students; of these, 28 have no other teaching responsibilities. The remaining departments have teaching obligations to a variety of combinations of dental, pharmacy, nursing, physical therapy, and dental hygiene students. Other students who are taught by some of the departments include occupational therapy students, residents, biomedical engineers, and undergraduate students in the arts and sciences. Fifty-eight of the 72 responding physiology departments are at institutions operating under the traditional program based on departmental courses. Fourteen departments are part of an integrated program involving at least one other department.

Significance of and Methods for Conducting a Laboratory Program

Anatomy Laboratories

Of the departments supplying complete information in gross anatomy, 27 spend between 25 and 50 hours in lecture, and 25 spend 50 to 100 hours. The mean is 50 hours of lecture; two departments spend less than 25 hours in the lecture portion of the course and four are in the 100–150 hour range.

In microscopic anatomy the mean time devoted to lectures is 41 hours with 38 departments ranging between 25 and 50 hours and 16 departments teaching between 50 and 100 hours. One department has a lecture time of less than 25 hours. In neuroanatomy the mean time allotted for lectures is 37 hours with two departments in the 0–25 hour range and seven departments in the 50–100 hour category. The majority of 48 groups spend between 25 and 50 hours in lectures.

Fifty percent, 69%, and 72% of the anatomy departments feel that the time which they have allotted for the laboratory phase in gross anatomy, microscopic anatomy, and neuroanatomy (respectively) is adequate to meet the objectives of the laboratory. Thirty percent feel that the time allotted for gross anatomy is less than adequate, 20% believe the same of microscopic anatomy, and 23% could use more time in the teaching of the neuroanatomy laboratory. More than adequate time was reported by 11% in gross anatomy, 9% in microscopic anatomy, and 5% in neuroanatomy. The mean time spent in the laboratory is 180 hours in gross anatomy, 80 in microscopic anatomy, and 59 in neuroanatomy.

The departments vary greatly as to the number of hours which are devoted to laboratory teaching. Some persons apparently feel very strongly, especially in gross anatomy, that laboratory programs are tremendously meaningful and important while others express doubt regarding their value. Ten departments spend between 0 and 125 hours in the gross anatomy laboratory, 29 teach between 125 and 225 hours, 7 are in the 225 to 300 hour range, and 1 department spends more than 350 hours in the gross anatomy laboratory. In microscopic anatomy 6 departments are in the 0–5 range, 16 in the 50–75 group, 17 in the 75–100 range, 12 in the 100–125 hour range, and 3 devote between 125 and 150 hours to the laboratory portion. In neuroanatomy, 19 departments teach 0 to 50 hours, 23 teach 50 to 75 hours, 11 teach 75 to 100 hours, while 3 departments spend 100 to 125 hours in the laboratory.

The distribution of the number of students per faculty member in a typical laboratory has a mean value of 24 in gross anatomy, 25 in microscopic

anatomy and 27 in neuroanatomy. When the student/faculty and graduate assistant ratio is considered, the value shifts to 19 for all three courses.

Laboratory attendance is required in 75% of the departments, and accordingly (Fig 1) in gross anatomy an average of 42% of the student's final grade is derived from his laboratory performance; the figures are 39% and 35% for microscopic and neuroanatomy courses respectively.

In gross anatomy 22 (42%) departments count practicals as 50 to 90% of the final grade while 13 (24%) use practicals as the sole measure of a student's accomplishments. In microscopic anatomy and neuroanatomy the figures for the 50–90% range are 32 (52%) and 24 (35%) respectively. Practicals are employed as the only evaluation of students in 12 (22%) microscopic and 14 (26%) neuroanatomy courses. Oral examinations are used by very few departments in all three disciplines for the evaluation of the students' performance. Written examinations, either as a separate examination or in conjunction with a lecture examination, seem to be quite popular since they are used by approximately 61% of the departments in all three subject areas. Written or oral laboratory reports are used in one department to aid the evaluation of the students' performance. Another method mentioned for evaluation of students' laboratory progress is a subjective evaluation of the students by the faculty either in the laboratory or in conferences and discussions about the laboratory work.

Flexibility in the curriculum for long or short laboratory periods as needed was reported in 43 (75%) of the departments while in 15 (25%) this possibility did not exist. The most popular length for any gross laboratory period is 3 to 5 hours although shorter and longer labs are often used. Laboratories in microscopic anatomy and neuroanatomy utilized periods of less than 3 hours to the greatest extent.

The three most esteemed objectives of laboratory exercises in all three courses in their order were (Figs 2a, b, c): *supplementation and reinforcement of didactic material, interpretation of clinical laboratory findings,* and *appreciation of experimental development and methodology.* The latter is essentially an appreciation of the "scientific method." Of less importance were the objective of *acquirement of manipulative skills* and *development of student-faculty relations.*

As can be seen in Figs 3a, b, and c, demonstrations and class discussions were used by about one-third of the departments and reflected 10% to 20% of total class time. Research projects were not employed and student conduction of pre-assigned experiments was also extremely rare in the three anatomy specialties. The mean percentages of lab-

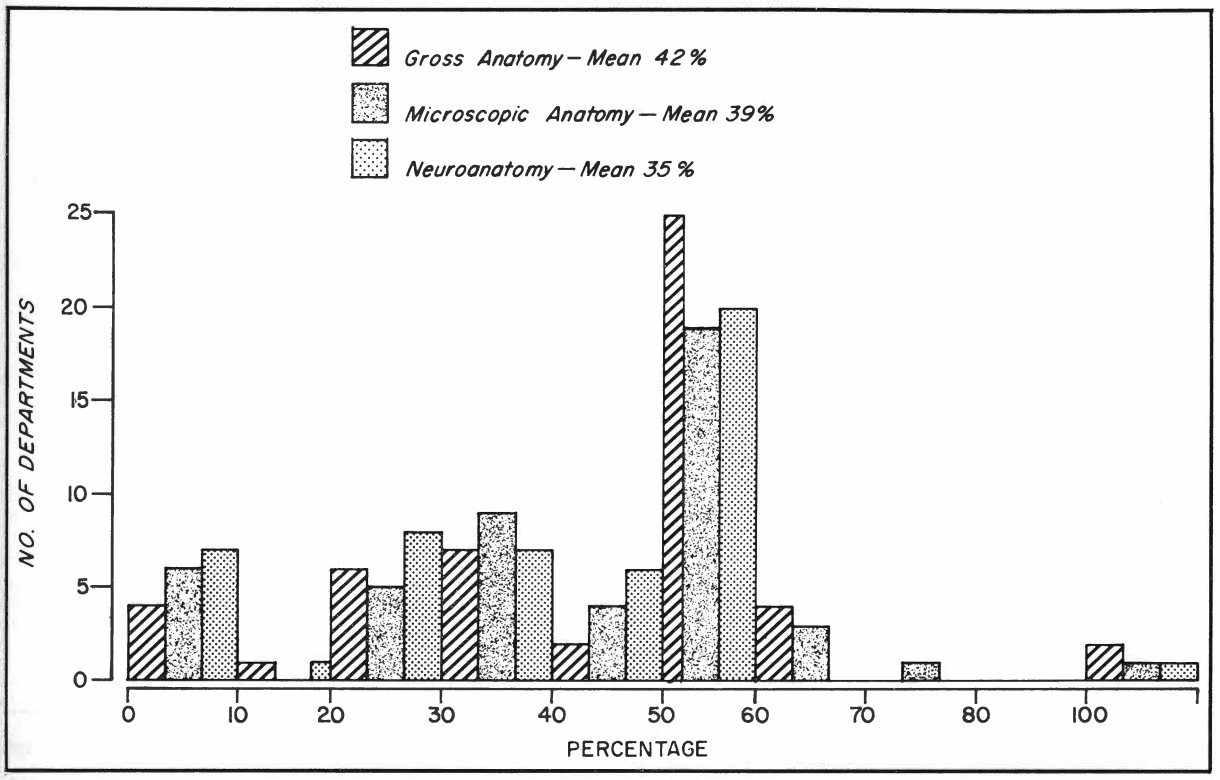

Fig 1—Distribution of percentage of the student's final grades derived from his laboratory performance.

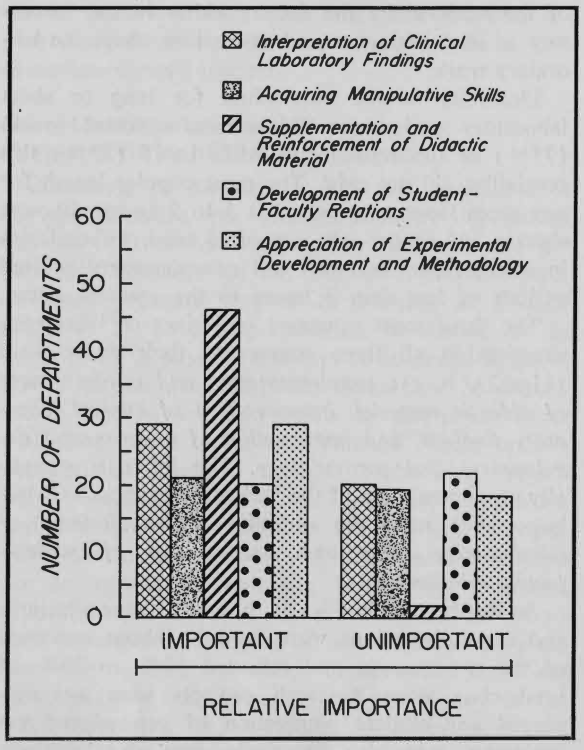

Fig 2(a)—Distribution of opinions concerning the importance of certain objectives of a gross anatomy laboratory program.

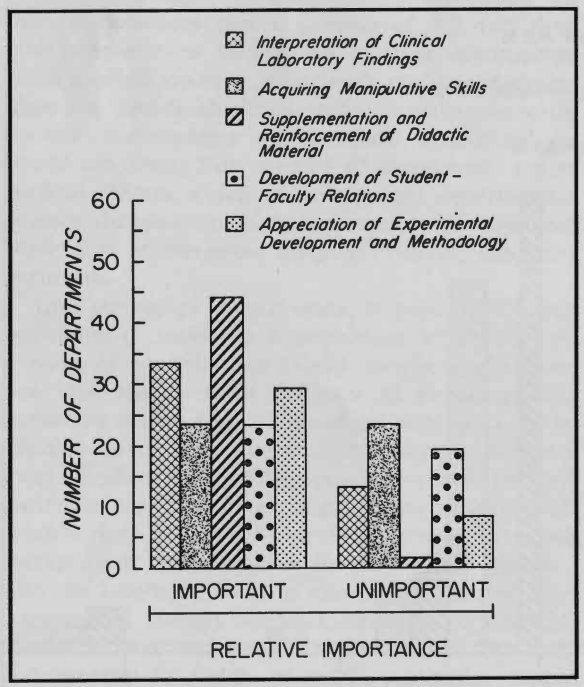

Fig 2(b)—Distribution of opinions concerning the importance of certain objectives of a microscopic anatomy laboratory program.

oratory work carried out by the students were 90% in gross anatomy, 80% in microscopic anatomy, and 82% in neuroanatomy; demonstrations in these three areas, respectively, comprised 8%, 10%, and 14% of the time.

The prevalent opinion of anatomists throughout the country seems to be that anatomy is a visual and manual science. Dissection and microscopic examination of material and painstaking repetition are still the most valuable assets to the student.

There is relative agreement that while planning effort and budget for the laboratory program have either remained unchanged or increased over the past five years, the actual laboratory time has either remained unchanged or decreased during the same period. Fifty-six percent of the departments have experienced a decrease in their time allotted for gross and microscopic anatomy; for neuroanatomy the corresponding figure is 42%. An increase for the anatomy disciplines was reported by 17% of the departments; the remainder report no drastic change in the time used for laboratory teaching.

Seventy-two percent used original microscopic slides in microscopic anatomy 90–100% of the time

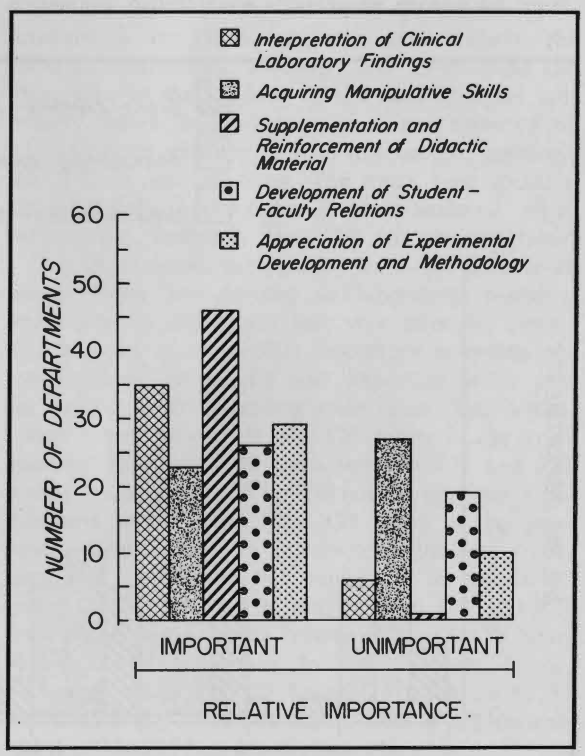

Fig 2(c)—Distribution of opinions concerning the importance of certain objectives of a neuroanatomy laboratory program.

while 59% do the same in neuroanatomy. Kodachrome projection slides are used between 0% and 20% of the laboratory time by 73% of the microscopic anatomists and by 64% of the neuroanatomists. Seventy-eight percent of the departments feel that the published laboratory manuals in the three anatomy disciplines do not meet the requirements and needs of the students and faculty. Of the responding departments 59% feel that some use of special visual aids (including films, tapes, and closed circuit television) improves student learning. Thirty-four percent of the departments feel that these visual aids shorten the time required for laboratory teaching while 56% feel the required time is unchanged but may be more effective. Ten percent think visual aids increase the time required for laboratory programs.

Most departments (90%) indicated that they use live black and white television (no one that replied indicated color capability), tapes and films, projections, predissected materials, and charts and models to a certain extent. In general, however, no more than 10% of laboratory programs are occupied by audio-visual aids. The basic teaching goals of 75% of the anatomy departments have, despite the current stress on change, remained the same; they are still interested in giving the student a well rounded and fairly complete course in the basic anatomy disciplines which will be a foundation for his future clinical training.

Several comments include the sentiments that "it is just plain stupid to cut hours in all courses to the same degree. Such a maneuver emasculates a course in Anatomy but does relatively little harm to Biochemistry and Physiology." Many people feel that "the trend to reorganize anatomy with a view to preparing specialists only is shortsighted and dangerous; there is a need for a family-type practitioner, which precludes the dangerous shortening of basic science material." The sentiment also voiced quite frequently points out a basic contradiction in our system of revising and changing curricula. At a time when more elective courses are being introduced, many decry the short span of four years available for medical education.

The feeling that "Anatomy is a laboratory subject; . . . (especially) so with Gross Anatomy, least with Neuroanatomy and with Histology in an intermediary position" is held by virtually everyone. "It is a visual science and as such reading textbooks gets one only partially and incompletely on the way to its understanding."

Several comments include the sentiments that "nothing replaces a high ratio of instructors to students," that "audiovisual aids are essential for small staffs with large numbers of students" and that

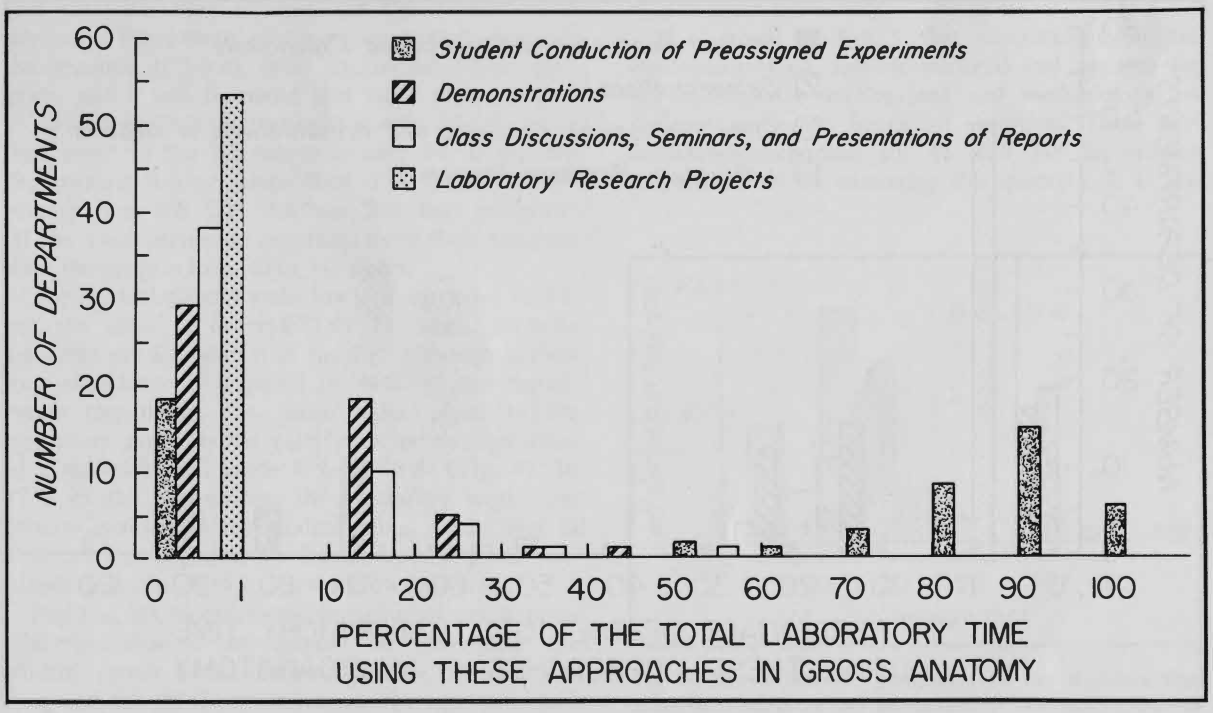

Fig. 3(a)—Distribution of approaches used in conducting the laboratory program.

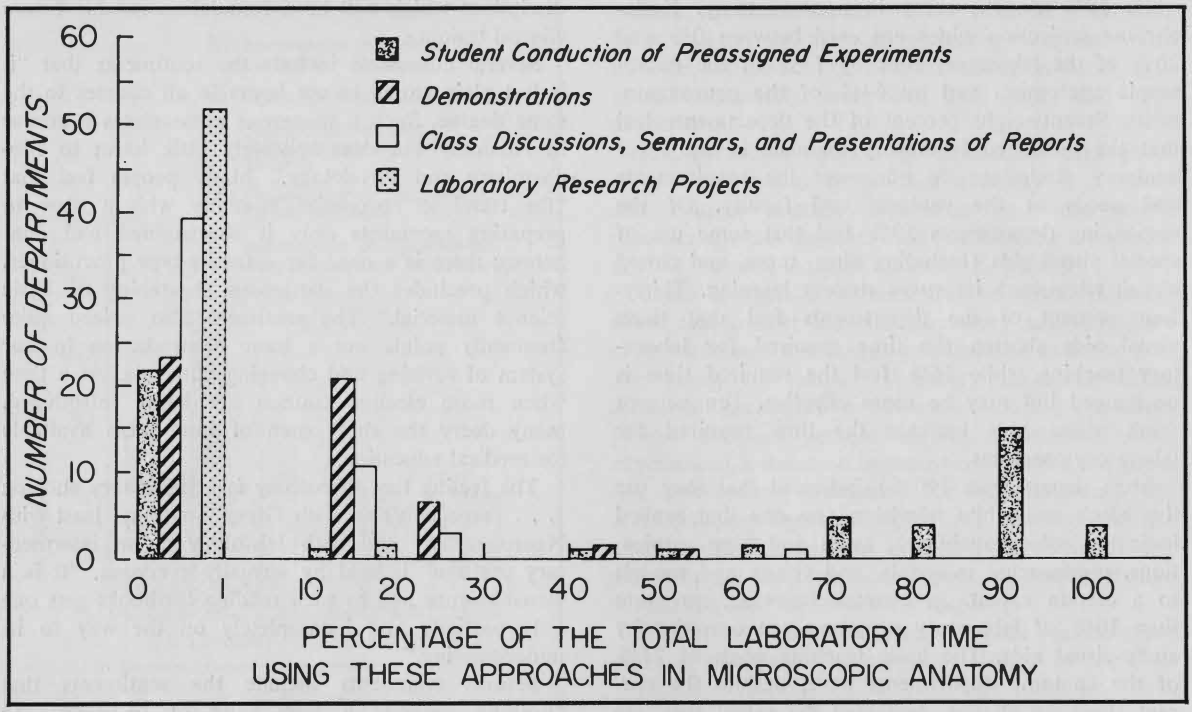

Fig 3(b)—Distribution of approaches used in conducting the laboratory program.

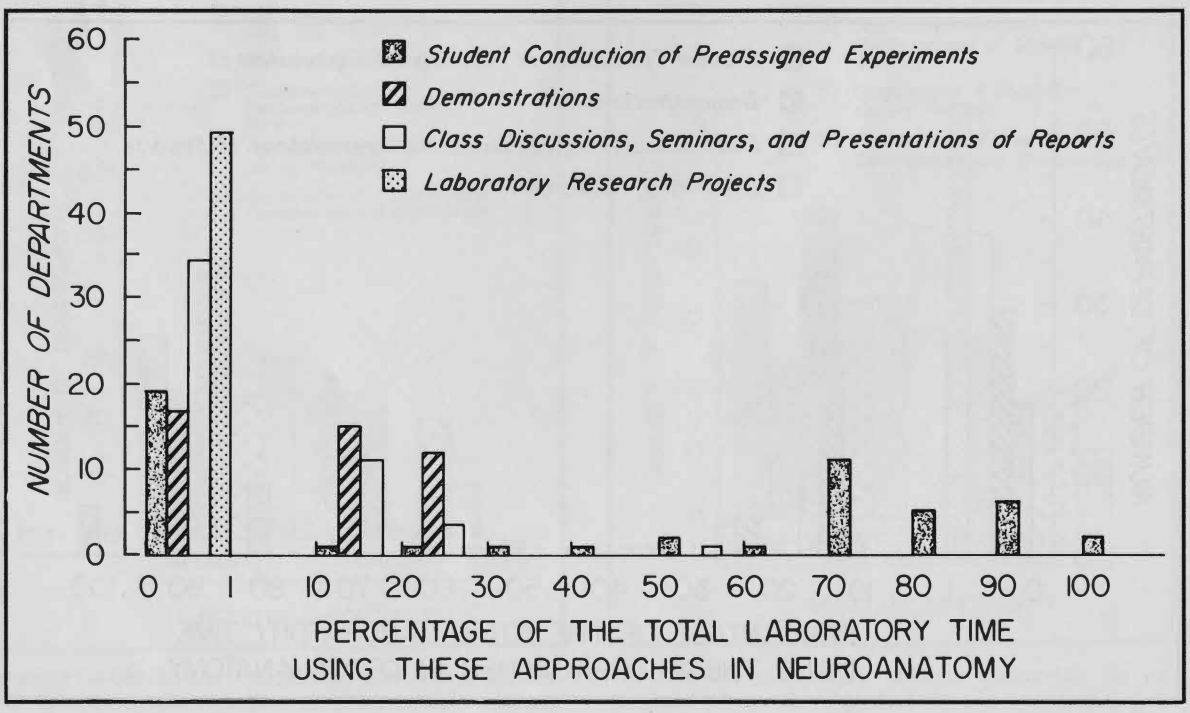

Fig. 3(c)—Distribution of approaches used in conducting the laboratory program.

"supervised dissection, predissected cadavers, chalk drawings and explanatory lectures are absolutely mandatory if the student is to gain any lasting value from the laboratory courses."

The feeling seems to be universal that a change in time and emphasis was needed; people feel that "tests are more reasonable in length and emphasis" and that the "attitude of the faculty is fairer" now. The opinion that an "M.D. needs a good general background" and that "repetition contrary to the thoughts of many is essential" is widely held.

The idea that "we should fight for maintaining our Gross Anatomy courses and should integrate Micro and Neuro" is widely held, but some people voice that the "integration scatters histology too much since the lectures and laboratories occur sporadically through the whole year." One could agree probably with the statements "deemphasize lectures, the lab is more important"; however, "it needs clinical orientation to a degree." "Laboratories should be made a learning experience instead of a teaching experience" expresses the sentiments of most anatomy departments.

Biochemistry Laboratories

Of 65 biochemistry departments reporting, 46 spend between 50 and 100 hours in lectures while 5 spend less than 25 hours. In the laboratory teaching program (including demonstration) there is a mean of 94 hours. The individual values range from 0 to 348 hours. Of 65 departments reporting, 44 feel that the time allotted for the laboratory is adequate. Forty-three of these departments reported the number of hours spent in the laboratory program, and it will be noted that these range from 0 to 304 hours. It is interesting that although the mean time spent in the laboratory is only 94 hours, two departments having more than 175 hours of laboratory time felt that this was less than adequate. Of the 14 departments reporting more than adequate time, the range is from 40 to 348 hours.

The student/faculty ratio has a mean value of 23, but the ratio is decreased to 13 when graduate assistants are included with faculty. Although laboratory attendance is required in 84% of the departments responding, the mean value given to the laboratory grade in the calculation or determination of a student's final grade is only 18% (Fig. 4). In 17% of the departments the laboratory work contributes nothing to the student's final grade, and in 58% of the departments the laboratory work contributes less than 20% to the student's final grade.

Eight of 63 biochemistry departments use a practical examination in helping to determine the student's grade in the laboratory; three of these give it credit for 50% or more of the student's laboratory grade. Oral examinations are used by only four departments. Written examinations are used in helping to determine the student's laboratory grade in 29 departments, and in 21 departments, written examinations comprise 50% or more of the student's laboratory grade.

Written laboratory reports assume somewhat greater importance in the evaluation of the student's laboratory grade. Only 16 of 63 departments give no weight to written laboratory reports; in 40 departments they comprise 20% or more of the student's laboratory grade. Indeed, in seven departments, the total laboratory grade is derived from this aspect of the student's performance. Oral laboratory reports assume somewhat less importance, but in 4 departments out of 63, they comprise 50% or more of the student's laboratory grade. This method of grading is not used at all in 53 departments. Other methods of determining the student's laboratory grade are used to some extent in 22 departments. In 11 of these, other methods determine 50% or more of the student's laboratory grade. (The majority of these involved an unspecified subjective evaluation of the student's laboratory performance. Some departments also mentioned grading of analytical results on laboratory unknown samples, seminars, and laboratory technique.)

Sufficient flexibility of curriculum to allow longer or shorter laboratory periods is reported in 40 of 64 departments. The laboratory of 3 to 5 hours length is still the most popular, but it may also be noted that seven schools use laboratories longer than 5 hours exclusively.

It is noted in Fig 5 that supplementation and reinforcement of didactic material and appreciation of experimental development and methodology are the most uniformly important objectives. These were considered important by 48 and 50 departments respectively of 66 answering this question. It is per-

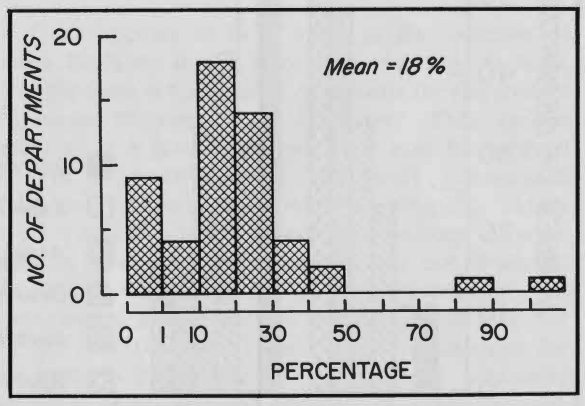

Fig 4—Distribution of percentage of the student's final grade derived from his biochemistry laboratory performance.

haps surprising that interpretation of clinical laboratory findings is considered quite unimportant. Experience and responsibility of working with live animals or tissues is similarly rated unimportant. The objective of development of student-faculty relations is considered to be an important objective in slightly more than half the departments.

Of 66 departments responding to this question, 55 use student conduction of pre-assigned experiments for a portion of their laboratory program (Fig 6). Although 46 departments spend 50% or more of their laboratory time in student conduction of pre-assigned experiments, no school uses 100% of its laboratory time for this purpose. Forty-eight use demonstrations in their laboratory program, but only six use this in more than 50% of their laboratory program. Two schools are noted to use demonstrations exclusively. Forty-one departments use class discussions, seminar, and presentation of reports for at least a portion of the laboratory program, but in only four departments does it amount to more than 30% of the laboratory time. Twenty-six departments use laboratory research projects in their laboratory program. Of these 26, 15 allot this approach 30% or more, and 8 departments use this approach for greater than 70% of the laboratory time. Since supplementation and reinforcement of didactic material is often considered an important objective of the laboratory program, the departments were asked which approaches they considered to be most effective in achieving this objective. Twelve of 41 departments selected student conduction of pre-assigned experiments, and 13 of 41 departments selected class discussion, seminars, and presentation of reports to be most effective. Laboratory research projects, and demonstrations were considered much less effective in reaching this particular objective.

Although the time devoted to the laboratory program was not reported to have increased in any of the responding schools, planning effort has increased in 35 and remained unchanged in 10 of 59 departments responding. In the past five years the budget has increased in 30 and remained unchanged in 19 of 57 departments reporting.

The use of visual aids is considered by 28 of 33 departments to improve student learning. At the same time 14 of 27 departments consider that the time required for laboratory teaching would be unchanged and 3 departments indicate that the time required for laboratory teaching would increase with use of visual aids. Only 4 of 58 departments make any use of live, closed-circuit television in laboratory teaching, and none utilize color.

Having presented tabulations of ordered responses, we would be remiss if we did not also report anonymously some remarks made in a section of the questionnaire requesting comments. One respondent stated tersely, "Biochemistry does not exist apart from the laboratory. Medical students may not like biochemistry laboratory. They need it to become acquainted with biochemistry."

On the other hand, one responded, "I am beginning to question seriously in my own mind the role of a laboratory in biochemistry to freshmen medical

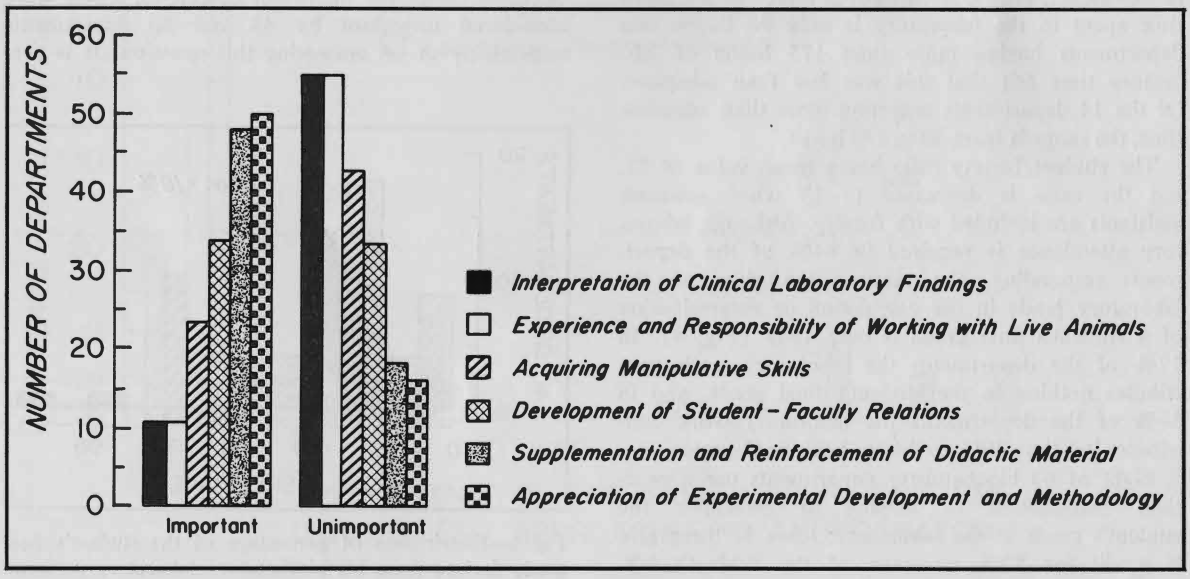

Fig 5—Distribution of opinions concerning the importance of certain objectives of a biochemistry laboratory program.

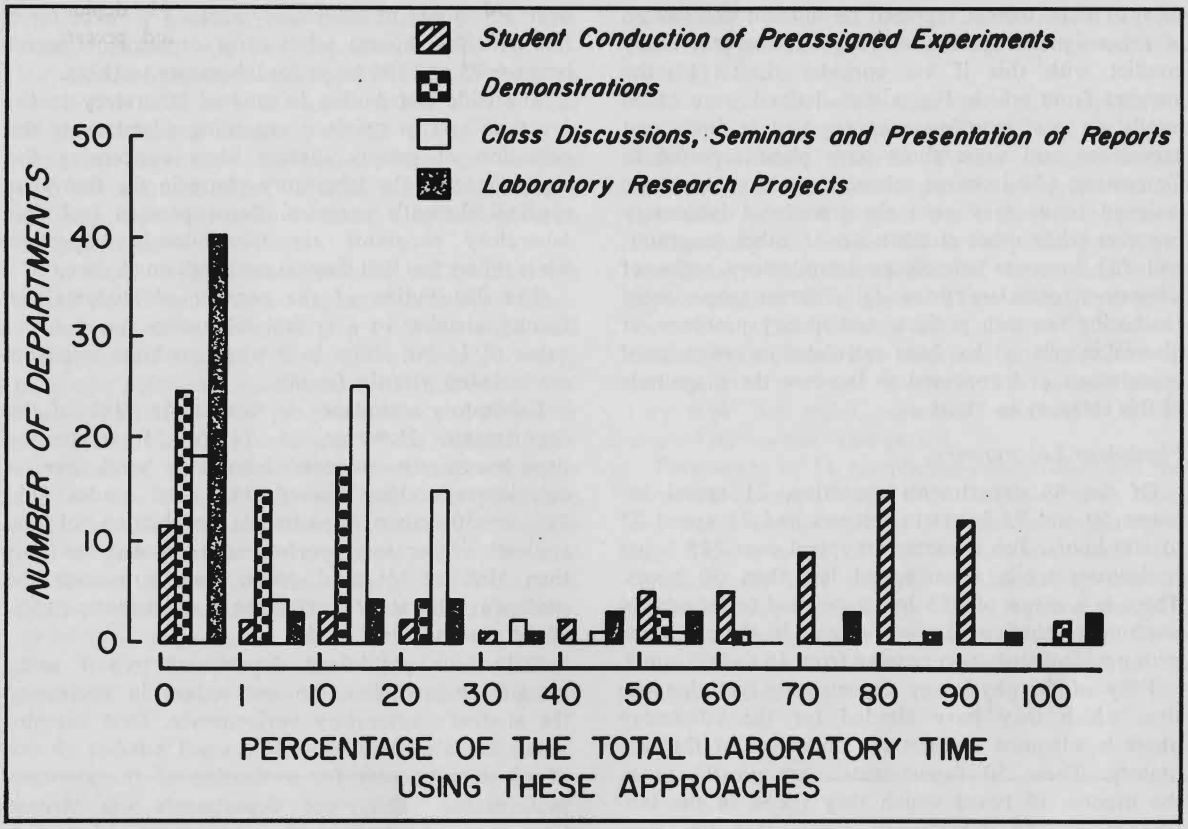

Fig. 6—Distribution of approaches used in conducting the biochemistry laboratory program.

students in general. For the relatively large investment in time involved I am not convinced that the student derives very much about biochemistry for his investment." Another says that "Our own staff is split . . . on [the] value of continuing any lab training in biochemistry." The view is also reported that ". . . the first year medical students dislike the lab as does the faculty."

Still another reports that, "It is becoming increasingly apparent to us that the laboratory program is the most difficult part of the course to make meaningful to students." And, questioning the need again, "Of the 10% [of the students] essentially opposed to laboratory experimentation, some have excellent backgrounds and do very well as measured by examination."

> One responded at some length, "It is our feeling that the laboratory for first year medical students should be constructed to emphasize problem-solving and introduction to the experimental method. We do not attempt to make research biochemists of these students but to introduce the concept that the practice of medicine involves asking scientific questions and interpreting data. Although the 'cook-book' approach is traditional, it does little to motivate, does not challenge, provides very little instructional benefit and renders the educational effort group-centered instead of individualized. The small group project approach makes it possible to give extra instructional effort for those who need it and to expand the scope of the project for those who are capable."

There appears to be a move in the direction of more flexibility in the laboratory program. At some schools there are a number of options for the student in regard to biochemistry laboratory. Thus various students in a given freshman class may be assigned to (or at some schools may elect) pre-assigned laboratory experiments, research projects, library work (with or without reports), or nothing. At some schools biochemistry laboratories are not available. In the *Comments* section of the questionnaire, 24 departments gave responses indicating that now (or in the next two years) there is no requirement for students to participate in a traditional laboratory program of pre-assigned experiments. When we consider that not all respondents addressed themselves to this particular problem in their comments, it seems safe to suggest this as a minimal figure. The fact that

only 11 departments reported no student conduction of pre-assigned experiments (Fig 6) does not really conflict with this if we consider that: (1) the answers from which Fig 6 was derived were based totally on past experience as opposed to both past experience and some short term plans reported in Comments; (2) in some schools certain students are assigned to or may elect the traditional laboratory program while other students are in other programs; and (3) in some schools an introductory series of laboratory exercises prior to different experiences (including research projects and library problems or discussion groups) has been calculated as pre-assigned experiments and appeared to increase the magnitude of this category in Fig 6.

Physiology Laboratories

Of the 68 departments reporting, 21 spend between 50 and 75 hours in lectures and 25 spend 75 to 100 hours. Ten departments spend over 125 hours in lectures while seven spend less than 50 hours. There is a mean of 113 hours devoted to laboratory teaching (including demonstrations) in these courses with a wide distribution ranging from 16 to 360 hours.

Fifty of 66 physiology departments feel that the time which they have allotted for the laboratory phase is adequate to meet the objectives of the laboratory. These 50 departments vary greatly as to the number of hours which they spend in the laboratory. Fifteen departments report that less than 75 hours is adequate while 13 have over 150 laboratory hours and feel that this is the appropriate amount. Of the five departments which feel their laboratory time is less than adequate only one had over 100 hours of laboratory teaching. Of the ten which feel the time they have allotted for the laboratory phase is more than adequate, seven had over 100 hours of laboratory teaching (two of these had over 200 hours) while three departments spend between 75 and 100 hours for laboratory teaching.

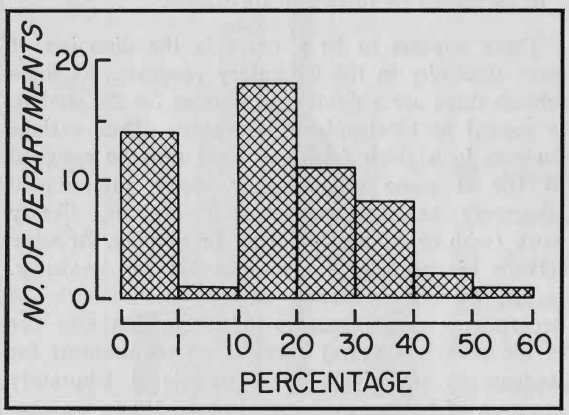

Fig 7—Distribution of percentage of the student's final grade derived from his physiology laboratory performance (Mean = 16%).

The wide distribution in allotted laboratory teaching time and in opinions regarding adequacy is the reflection of greatly diverse ideas concerning the significance of the laboratory phase in the first year medical student's program. Some persons feel that laboratory programs are tremendously important while others feel that they do not merit much time.

The distribution of the number of students per faculty member in a typical laboratory has a mean value of 16 but shifts to 9 when graduate assistants are included with the faculty.

Laboratory attendance is required in 71% of the departments. However, in 14 of 55 responding departments the students' laboratory work *per se* contributes nothing toward their final grades (Fig 7); in 30 other departments, evaluation of the student's laboratory performance contributes less than 30% of his total grade. On the average the student's laboratory performance contributes 16% of the student's final grade.

Only four physiology departments report using practical examinations to any extent in evaluating the student's laboratory performance. Oral examinations are also used by only a small number (8 out of 63 departments) for evaluation of the student's performance. Thirty-one departments use written laboratory examinations to some extent either as a separate examination or in conjunction with a lecture examination. Written examinations seem to be the most popular method for evaluating the student's performance.

Nine departments use oral laboratory reports to help evaluate the student's performance while 23 departments use written laboratory reports for this purpose. In four cases these reports make up the entire laboratory grade and in 15 other departments they contribute to 50% or more of the final laboratory grade. Another method mentioned for evaluation of the student's laboratory performance is a subjective evaluation of the students by the faculty either in the laboratory or in conferences and discussions about the laboratory work. Mentioned also as influencing the laboratory grades in some departments are projects, electives, attendance and term papers.

Flexibility for having long or short laboratory periods as needed is reported in 55 of 69 departments. The most popular length for a lab period is 3 to 5 hours although shorter and longer labs are being used considerably. Twelve departments use lab periods over 5 hours long exclusively.

The two most esteemed objectives for a laboratory program were supplementation and reinforcement of didactic material and an appreciation of experimental development and methodology (Fig 8).

The latter is essentially an appreciation of the "scientific method." Of less importance were the objectives of acquiring manipulative skills, interpretation of clinical laboratory development and development of student-faculty relations. As for the objective of obtaining experience and responsibility of working with live animals, 38 of 72 cases rated this objective as being important while 34 rated it as having much less importance.

Of the 69 departments responding, 62 used student conduction of pre-assigned experiments to some extent in their laboratory program (Fig 9). Forty-eight used this approach in greater than 50% of their laboratory programs. Fifty-one departments conducted demonstrations as part of their lab programs; however, they were used for only a small percentage of the total lab time. Forty-two of these departments used demonstrations for less than 30% of the total time. Forty-five have class discussion, seminars and presentation of reports also as part of their lab programs. As in the case of demonstrations the class discussions occupy a relatively small percentage of the total laboratory time; 39 of these departments had class discussion in less than 30% of their lab programs. Twenty-three departments use laboratory research projects to some extent in their lab programs. Of the 23, 14 use it for less than 30% of the time while 3 use it for more than 70% of the total lab time.

There are diverse opinions on which approach is most effective for supplementation and reinforcement of didactic material. Twenty thought demonstrations made the best approach while ten thought laboratory research projects made the best approach. Intermediary importance for accomplishing this goal was given to class discussions and student conduction of pre-assigned experiments. There is relative agreement that while planning effort and budget for the laboratory program has remained unchanged or increased over the past five years the actual laboratory time has either remained unchanged or decreased during this same period.

Forty-eight of 54 responding departments feel that some use of special visual aids (including films, tapes, and closed circuit television) improves student learning. Twenty-seven of 53 think these visual aids shortened the time required for laboratory teaching while 23 departments feel the required teaching time is unchanged but may be more effective. Three think visual aids increase the time required for laboratory teaching.

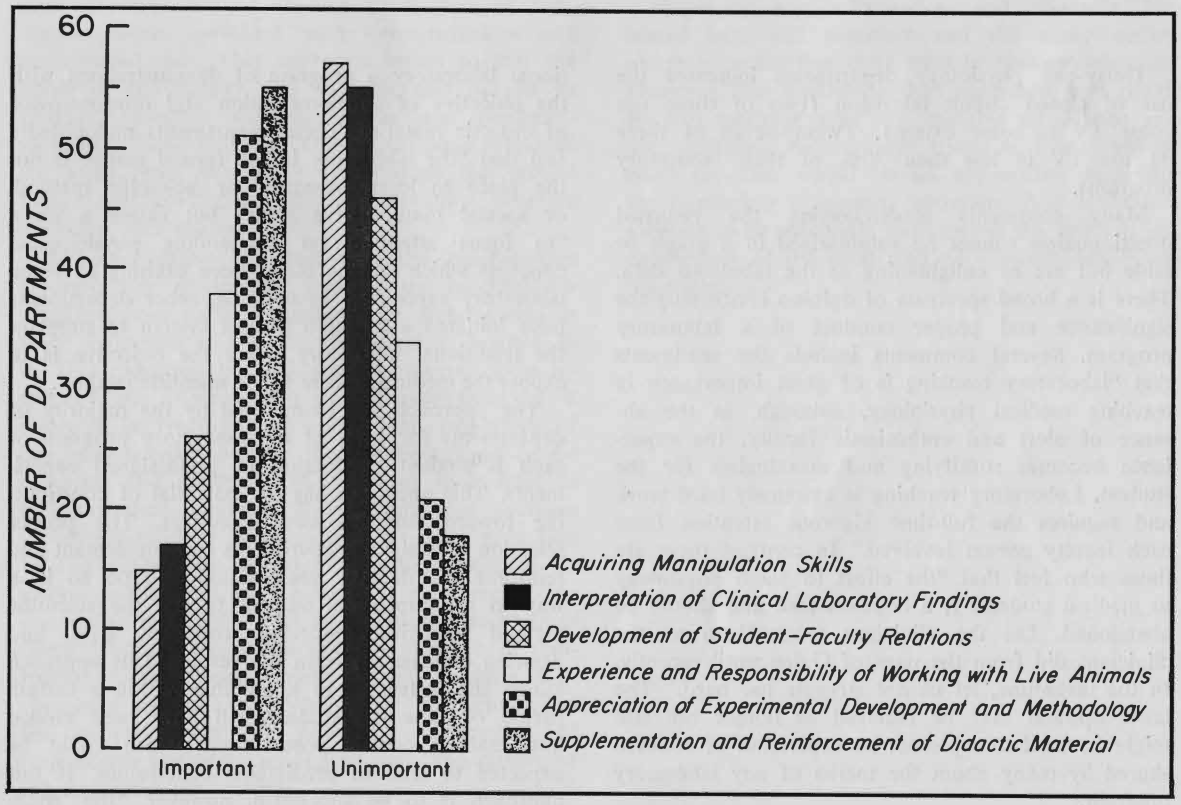

Fig. 8—Distribution of opinions concerning the importance of certain objectives of a physiology laboratory program.

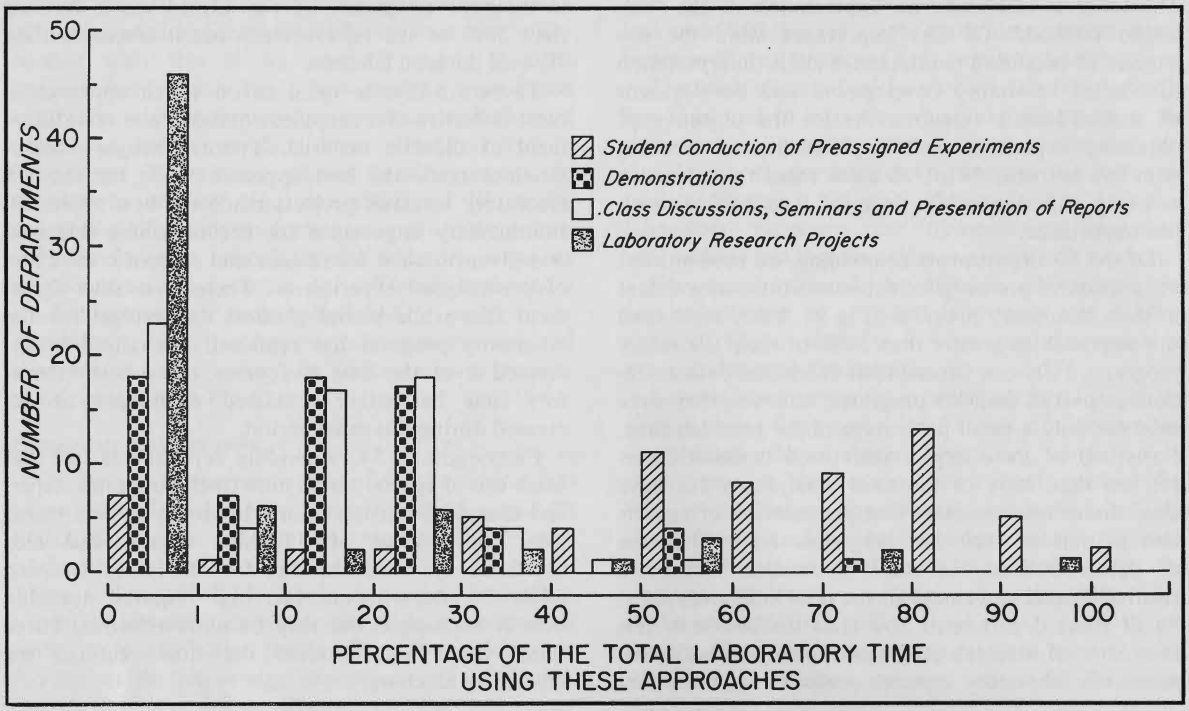

Fig. 9—Distribution of approaches used in conducting the physiology laboratory program.

Thirty-one physiology departments indicated the use of closed circuit television (two of these use color TV to some extent). Twenty-seven of these 31 use TV in less than 30% of their laboratory programs.

Many comments accompanying the returned questionnaires cannot be summarized in a graph or table but are as enlightening as the tabulated data. There is a broad spectrum of opinion concerning the significance and proper conduct of a laboratory program. Several comments include the sentiments that "laboratory teaching is of great importance in teaching medical physiology, although, in the absence of alert and enthusiastic faculty, the experience becomes stultifying and meaningless for the student. Laboratory teaching is extremely hard work and requires the full-time vigorous attention from each faculty person involved." In contrast there are those who feel that "the effort to teach physiology to medical students is a hopeless task and should be abandoned. Let the clinicians teach them as the clinicians did from the days of Galen until recently. In the meantime, let us not struggle too hard." The latter opinion may be received as humor but still might be used to represent an expression of concern shared by many about the merits of any laboratory program.

Some departments have substituted for the traditional laboratory a program of demonstrations with the objective of supplementation and reinforcement of didactic material. These departments undoubtedly feel that "the laboratory in the formal course is not the place to learn 'research' or 'scientific method' or special manipulative skills" but rather a place "to focus attention on outstanding physiological concepts which can be made more exciting by some laboratory exposure." In contrast, other departments have initiated a research project system to supplant the traditional laboratory. Here the objective is to expose the medical student to the scientific method.

The approach still being used by the majority of departments for most of the laboratory program of each is student conduction of pre-assigned experiments. This approach has the potential of contributing toward fulfilling two objectives. The proper selection of assigned experiments can supplement and reinforce the didactic presentation and do so in a way to encourage the student to use the scientific method involving observing, collecting data, and drawing conclusions from the results. This approach allows the instructor to know that within a certain period of time the student will have been guided into making certain observations which could be expected to lead to predictable conclusions. If this approach is to be successful, however, "the 'cookbook' experiments must be carried out in the mind

as well as by hand. The student will take the latter course if left to his own devices, hence the importance of active pursuit of the important principle by the instructor."

Opinions were also given concerning factors enhancing the students' enthusiasm and curiosity in the laboratory. These include assurance of "some perceivable clinical or career relevance in material presented" and insistence that "trivia must not be emphasized simply because they are readily recorded."

Summary

The laboratory in the anatomy disciplines is regarded as the cornerstone and foundation of the dissemination and mastering of the basic knowledge of anatomy. Laboratory time has been reduced in almost all instances and especially in gross anatomy. Histology courses still are satisfactory and the integration of neuroanatomy has been welcomed. These changes have occurred either by design or necessity. A moderate reduction in time in most cases has not produced inadequacies in the laboratory according to a subjective evaluation by the departments involved, but most would prefer a yardstick to measure the effectiveness of the changes. The majority of anatomy departments have, at least to the present time, retained the basic traditional laboratory program centered around student conduction of regimented work, sprinkled with demonstrations and audio-visual aids. This approach seems to suit the needs of the faculty and students and has as its primary objective supplementation and reinforcement of didactic material. These goals have remained the same from year to year although the precise nature and conduction of the laboratory program has often been changed.

Although many still feel that the biochemistry laboratory is an integral part of the biochemistry experience for medical students, the trend seems to favor reduction in the amount of time devoted to it. This reduction has not always occurred as a result of the biochemistry departments thinking it desirable in teaching, but more than two-thirds of our respondents consider their present time to be adequate and another 23% consider their time to be more than adequate. In addition to a decrease in laboratory time there appears to be a reduction in the percentage of laboratory time devoted to student conduction of pre-assigned experiments. As one individual says, "It is my opinion that conventional laboratory exercises are not effective in reinforcing lecture presentations or in teaching biochemistry methods or techniques." In deciding on the proper approach, however, a number of respondents sought to remind us that "the quality of the students is very critical."

The majority of physiology departments have retained in their medical physiology courses the traditional laboratory program centered around student conduction of pre-assigned experiments and sprinkled with demonstrations, conferences, and seminars. However, several physiology departments have reduced the time allotted to this laboratory work and their evaluation is that no inadequacies have developed. Some departments have substituted research projects for the traditional laboratory program indicating the importance they place on teaching the scientific method. Other departments have adopted a laboratory program centered around demonstrations designed to illustrate physiological concepts which either supplement or reinforce the didactic presentations.

The professional educators tell us that we must define the objective and be able to measure the degree to which the objective is reached in effective teaching experiences. It is clear that: (1) there is less agreement on the objective of the laboratory experience than might be desired, and (2) the degree to which the objective is reached is not easily measured. We feel the latter statement to be justified on the basis of our own experience in laboratory grading as well as the lack of emphasis of the laboratory grade in the student's final grade as compared to the relative time spent in the laboratory and lecture.

Hopefully the information and thoughts presented here will stimulate and aid many departments in evaluating their own laboratory programs. However, one contributor has cautioned, and rightly so, that teaching is a ". . . creative art. Hence, neither gathering statistics nor trying to mimic anyone else's program would be an appropriate technique for improving a teaching program. Any laboratory exercise to be successful must reflect the interests and convictions of the local staff as a worthwhile learning experience."

New Developments in Screening for Inborn Errors of Metabolism*

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Because the mental retardation caused by some inborn errors of metabolism is to a large degree preventable by appropriate and early therapy, consideration is now being given to the possibility of mass screening for these disorders in the neonatal period. Experience with the prototype, the phenylketonuria (PKU) program, dictates that mass screening for inborn errors of metabolism is here to stay and will most certainly expand in the future—like it or not. Since I have recently gained some insight into the field as Metabolic Consultant to the State Health Department, I wish to review the PKU program in Virginia—the many pitfalls in the diagnosis and treatment of this disorder, and the feasibility of mass or selective screening for other inborn errors.

Mass Screening

In 1966, Virginia passed a law making it mandatory to test all newborns for the presence of PKU; indeed, today most states have such a law. The State Health Department responded to the legislature's charge to implement the program by: 1) establishing accurate Guthrie testing in three State laboratories, 2) establishing a quantitative serum phenylalanine (PA) and tyrosine procedure at its central laboratory in Richmond, and 3) providing nutritional and medical follow-up for diagnosed cases. Three full years of experience with the program have demonstrated its worth not only financially, but also in the prevention of mental retardation.

From the bookkeeping viewpoint, at $0.42 per test, 97% of the 76,000 births in the State of Virginia last year were screened for about $31,000. At an incidence of one in 13,000 it thus costs approximately $6,000 to uncover one case; it costs another $2,000 to provide therapy. In contrast, the cost to the State for the lifetime institutional care of the invariably severely retarded and untreated PKU patient is, very conservatively, $125,000. One state's estimate put it at twice that figure. A recent review of the rolls of the State's institutions discloses 45 such PKU patients. At present we are actively treating 30 children at home, most of them diagnosed since the inception of the testing program. These compelling statistics should make believers of any nonbelievers regarding the financial soundness of the program.

But we must soberly admit what this program hath wrought, especially in the way of need for a more precise diagnosis and long term follow-up of positively screened patients. In the classical phenylketonuric, with markedly deficient PA hydroxylase activity (Fig 1), the serum level will reach greater than 20 mg % by the second week and usually 30–40 mg % by the third week. 0-hydroxyphenylacetic acid usually appears in the urine when the serum phenylalanine level reaches 7 mg %, but phenylpyruvic acid is not excreted in the urine until the serum phenylalanine reaches 13 to 15 mg %. Because this latter substance is what makes the ferric chloride test positive, it can be seen that urine tested under age 2–3 weeks might be negative despite a high blood level. It is for this reason that urinary screening gave way to blood screening via the Guthrie test. Of those who have a *permanent* elevation of serum PA (ie, greater than 3–4 mg %), approximately ⅔ will follow this course where dietotherapy is necessary to allay mental retardation (Berman et al, 1969).

Because phenylalanine is an essential amino acid it cannot be completely eliminated from the diet; the aim therefore is to reach that amount of PA intake which will maintain the serum level between 3 and 7 mg % (Berry and Wright, 1967). This amounts to approximately ⅙ the normal intake, or 50–80 mg/kg per day in early infancy and 20–40 mg/kg per day in older infants and children. Why maintain the serum level at 3–7 mg when the normal is 1–1.5 mg %? If one aims for that normal level too much danger of undershooting exists, and PA deficiency is almost as deleterious to the developing infant as hyperphenylalaninemia. In fact, it has caused death in an occasional infant (Davens et al, 1965). Presently there is a co-

* Presented at the Virginia Pediatric Society Meeting, Williamsburg, Virginia on February 27, 1970.

operative study underway to determine if the 3–7 mg % range might be relaxed to 10–12 mg % without deleterious effect, as there is no knowledge of what level correlates with injury to the developing central nervous system. These ranges of intake are only rough guidelines, and frequent monitoring of serum PA levels by Guthrie testing is required to determine the more exact individual requirements. To make matters more difficult, these requirements often change with varying protein needs induced by infection, changes in the rate of growth, activity, etc.

Variability of Phenylalanine and Protein Needs

Of the 15 cases of phenylketonuria currently being followed in the MCV Metabolic Endocrine Clinic, I have selected two which demonstrate these extremely variable phenylalanine and protein needs. *The first case* (Fig 2) is that of a premature baby with a birth weight of 4 lb at 32 weeks gestation. The child was referred to MCV at age 3 weeks weighing 3 lb 4 oz, and having a serum PA level of 38 mg % with a normal serum tyrosine and without detectable phenylpyruvic acid in the urine. Extreme lethargy, persistent vomiting, and abdominal distension were present on admission and were not ascribable to other causes (ie, sepsis, organic bowel disease, etc), and might have been due to the high serum PA level. For this reason an extremely restricted PA intake of 16 mg/kg per day was instituted and consequent to the fall in the phenylalanine level, clinical symptoms disappeared and weight gain ensued. However, with a rapid weight gain the PA needs markedly increased and even intakes of 80–90 mg/kg per day did not suffice to maintain the serum PA level above 2 mg %. For this reason it was thought that this case might merely be one of transient hyperphenylalaninemia and a full phenylalanine intake of 120 mg/kg per day was offered in the form of regular milk. This resulted in a rise of serum phenylalanine to 22 mg %. Therefore, an intake of 80–90 mg/kg was again used with a consequent fall in the serum PA again to less than 2 mg %. Regular milk supplying 160 mg/kg PA per day raised the level to 15 mg %, and despite a drop in intake to 100–120 mg/kg per day, it continued to rise to 28 mg %. This time a decrease in intake to 80–90 mg/kg brought the infant to the correct range. By this time the rate of growth had also slowed somewhat. Since discharge this child's serum PA level has been maintained between 3–7 mg % on an even more reduced intake of 50 mg/kg per day. This infant, then, with the catch-up growth typical of the premature, initially required considerably more than the expected amount of dietary PA (90–100 mg/kg per day).

The second case is that of a 9 year old girl with an I.Q. of 85 who had findings in early infancy of classical PKU (Fig 3). She was maintained in fairly

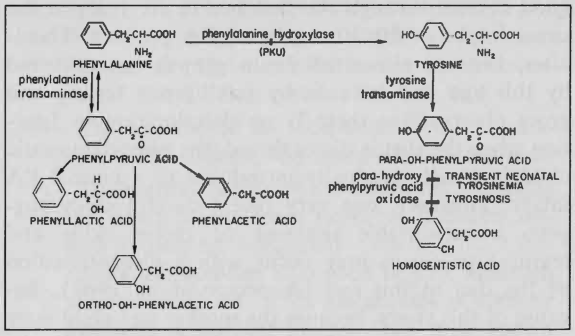

Fig 1—The classical phenylketonuric with markedly deficient PA hydroxylase activity.

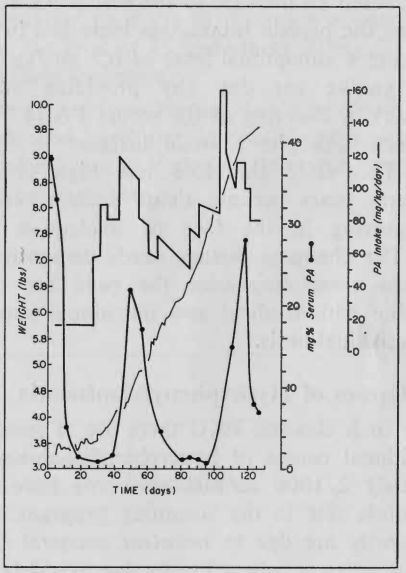

Fig 2—The course of case one.

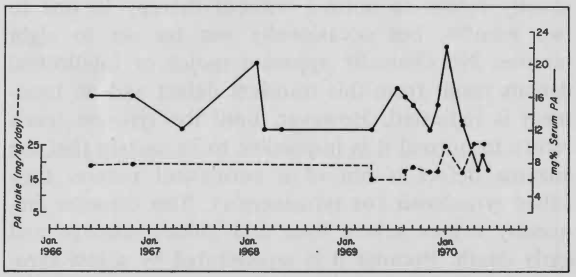

Fig 3—The course of case two.

good control through the first five to six years at the usual intake of 20–30 mg/kg of PA per day. Thereafter, because almost all brain growth has occurred by this age and because by intelligence testing and gross observations there is no deterioration in function when the diet is discontinued, the phenylketonuric child is usually gradually introduced to a normal PA intake. However, one very recent careful study suggests a measurable decrease of motor skills and learning processes may occur with a discontinuation of the diet at this age (Anderson et al, 1968). Because of this study, because the mother and child were most comfortable with the diet, and because the mother was fearful of any possible deterioration, PA restriction was continued. However, for the past three years, despite a low dietary PA intake of 16 mg/kg per day, the serum PA has been no lower than 12 mg %. An increase in dietary intake of PA (as natural food protein) to 24 mg/kg per day in December, 1969, merely caused an increase in the serum PA to 22 mg %. When the protein intake was increased in March, 1970, from a suboptimal level of 0.7 gm/kg per day to 1.3 gm/kg per day (by providing additional Lofenalac), a lowering of the serum PA to 7 mg % was effected, despite a small increase in total PA intake. This child therefore had high serum PA for several years because tissue protein breakdown was occurring in the face of inadequate protein intake. The changing dietary needs demonstrated by these two cases emphasize the need for constant monitoring with medical and nutritional supervision on an individual basis.

Other Causes of Hyperphenylalaninemia

Other than classical PKU there are at least five to six additional causes of hyperphenylalaninemia. Approximately 2/1000 normal newborns have a positive Guthrie test in the screening program, but the vast majority are due to *transient neonatal tyrosinemia.* This entity is believed to be due to a delay in the maturation in the liver of the enzyme parahydroxyphenylpyruvic acid oxidase, and large amounts of parahydroxyphenylpyruvic acid are usually found in the urine. Secondarily, serum PA rises and gives the positive Guthrie test, but serum tyrosine is characteristically much higher than phenylalanine. The values usually return to normal without therapy in one to two months, but occasionally not for six to eight months. No clinically apparent motor or intellectual deficits result from this transient defect and no treatment is indicated. However, until the tyrosine levels return to normal it is impossible to be certain that the enzyme defect is not of a permanent nature, then called *tyrosinosis* (or tyrosinemia). This disorder frequently causes severe liver and kidney damage and early death. Because it is ameliorated by a low tyrosine diet it becomes imperative that neonatally elevated tyrosine levels picked up by positive Guthrie tests be followed serially until they become normal.

Another cause of hyperphenylalaninemia of a transient nature is a *delay in the maturation of the enzyme PA hydroxylase* where the infant initially has classical findings of PKU but later in infancy tolerates a normal phenylalanine intake without hyperphenylalaninemia. Because of this possibility the dietary restriction of all infants with what initially appears to be classical PKU should be relaxed periodically to demonstrate persistence of the defect, as was done in my first example.

Of the approximate ⅓ of infants with permanent hyperphenylalaninemia who do not have the classical course of PKU, we encounter two generally described types. One is called *atypical PKU,* where levels of greater than 20 mg % are not associated with mental retardation despite there being no treatment. These children are usually uncovered as an older sibling of a newly diagnosed case from the screening program. The other term, simply *hyperphenylalaninemia,* is applied when the serum PA level never rises above 20 mg % without dietary restrictions and is usually not associated with mental retardation. The PA tolerance tests unfortunately do not distinguish these groups from the classical PKU patient, but there is some evidence to suggest that the absence in the urine of the breakdown products of PA, namely phenylpyruvic acid and orthohydroxyphenylpyruvic acid, favors a diagnosis of one of these milder forms of the disease. Indeed, some qualified investigators recommend not treating an infant unless there are PA metabolites in the urine, no matter how high the level of serum PA. It is therefore clear that we may be unnecessarily treating, with inherent dangers, as many as ⅓ of patients thought to have classical PKU. There is obviously a need for better means of diagnosing these subgroups.

Finally, there is the rare infant who lacks the enzyme phenylalanine transaminase but who only has hyperphenylalaninemia when on a higher than normal PA intake. In this instance, of course, no phenylpyruvic acid or orthohydroxyphenylpyruvic acid appears in the urine. Space does not permit a more detailed discussion of these variants, but I have mentioned them to emphasize that whereas just five years ago hyperphenylalaninemia was thought to be a simple disease state, mass screening has disclosed a wide spectrum of clinical and biochemical presentations with variable prognoses and treatment.

Indications for Screening

With some of the machinery already established, the next logical question deals with what other inborn errors should be screened. To answer this it would be best at this point to review the ten principles of early detection outlined by a World Health Organization team a few years ago (Wilson and Junger, 1968). It

will be seen that by these criteria the PKU program is a valid one:

1) "The condition should be an important problem." Although not terribly common, PKU is important as it is a treatable cause of mental retardation.
2) "There should be an accepted treatment for patients with recognized disease." There is for PKU. For other aminoacidopathies it is not so well worked out—for galactosemia, to be mentioned shortly, there *is* an accepted treatment.
3) "Facilities for diagnosis and treatment should be available." These do exist but are most marginally funded, so that the more detailed work-ups required with increasing awareness of the heterogeneity of the disease are not possible. This will be a drawback to establishing other mass or selective screening programs.
4) "There should be a recognizable latent or early symptomatic state." In the instance of PKU this is hyperphenylalaninemia, but at best this is likely an early symptomatic stage. Even dietary therapy instituted at less than 2 months of age results in only a mean I.Q. of 85 ± 12 vs. the unaffected sibling comparison of I.Q. of 109 ± 11. Whether the treatment is started too late or is inadequate is not known.
5) "There should be a suitable test or examination." The Guthrie test fits this bill well.
6) "The test should be acceptable to the population." The Guthrie test again fits this bill well.
7) "The natural history of the condition, including the development from latent to declared disease should be adequately understood." Classical PKU qualifies to a good degree, although what level of PA or its metabolites is actually injurious is not yet known—nor is it known why some patients with significant elevations of serum PA do not develop retardation, as in atypical phenylketonuria.
8) "There should be an agreed policy on whom to treat as patients." We are in the same trouble here with PKU because, as mentioned earlier, some physicians would delay treatment until metabolic products in the urine appear. Some use the level of 15 and others 20 mg % before starting treatment.
9) "The cost of case finding should be economically balanced in relation to possible expenditure on medical care as a whole." As pointed out, PKU scores high here.

TABLE

Urinary Screening Tests for Metabolic Defects

Disease	$FeCl_3$	DNPH	C–N	Benedict's	C–TAB
1. Phenylketonuria	Green	+++	−	−	−
2. Maple Syrup	Navy Blue	+++	−	−	−
3. Tyrosinosis	Trans. Green	+++	−	±	−
4. Histidinemia	Green	++	−	−	−
5. Hyperglycinemia	−	+++	−	−	−
6. Fructose Intol.	−	±(?)	−	++	−
7. Galactosemia	−	−	−	++	−
8. Cystinuria	−	−	++	−	−
9. Homocystinuria	−	−	++	−	−
10. Hurler's	−	−	−	−	++
11. Morquio-Ullrich's	−	−	−	−	±
12. Fanconi's	±	±	±	+	−
13. Alkaptonuria	Brown				
14. Lowe's		+			
15. Hyperlysinemia		+			

DNPH = dinitrophenylhydrazine
C–N = cyanide nitroprusside
C–TAB = cetyl trimethylammonium bromide
± = sometimes positive

10) "Case finding should be a continuing process and not a once and for all project." This is certainly true for PKU.

On the basis of these criteria, what other inborn errors would qualify for mass screening? Most aminoacidopathies probably do not qualify at this time mainly because of their rarity and of the expensive chromatographic techniques required, making case-finding too costly. Reduction in cost of detection might be achieved by the application of the bacterial "inhibition assay" technique as used in the Guthrie test. In this procedure the excessive leucine of maple syrup urine disease prevents the inhibition of B. subtilis by 2-methyl leucine, and excessive histidine of histidinemia prevents inhibition of B. subtilis by azaserine. With more refined resins and increased automation, quantification by ion-exchange chromatography might be a useful tool for mass screening of aminoacidopathies, but this development is several years away. In the meantime pilot studies in specialized centers are proceeding, using one or two dimensional paper or thin layer chromatography, or high voltage electrophoresis as screening tools. In this way more knowledge of these rare diseases and their treatment can be obtained and we can then better satisfy criteria 2, 7, and 8, listed above. One such recent study (Clow, Saiver and Davies, 1969) of the plasma of over 36,000 neonates revealed 316 with hyperaminoacidemia, but all except six were only transient. Five of the six permanent elevations were hyperphenylalaninemias and one was a case of hypermethionemia.

Considerations are somewhat different concerning the disorder of carbohydrate metabolism, *galactosemia.* In this disorder a buildup in the blood and tissues of galactose and galactose-1-phosphate causes mental retardation, cataracts, liver and kidney disease, and usually early death. Whereas previous estimates have put the incidence at 1:50,000, recent mass screening surveys put it at between 1:20,000 to 1:30,000 (Hansen, 1969). Various screening tests are presently being evaluated—measuring either blood galactose levels via a bacterial inhibition assay technique or the actual levels of the deficient enzyme, galactose-1-phosphate uridyl transferase, from spotted filter paper or with 50 microliters of heparinized blood. As for phenylketonuria and maybe for most inborn errors, it has already been established that there are several variants of galactosemia—four at present. It is not totally clear which types require therapy, but because this disease satisfies most of the established principles, with good treatment available (lactose elimination) it would appear that as soon as it is determined which test is most suitable, it will be the next metabolic disorder to be mass screened. Indeed one state has already made testing for galactosemia mandatory and several other states are seriously considering it.

There are a host of other inborn metabolic disorders for which diagnostic tests are available, but their consideration for inclusion in mass screening are not as pressing as the aforementioned diseases, either because of their rarity (Wilson's disease), the lack of adequate therapy, or the lack of a test acceptable to the population (cystic fibrosis).

Selective Screening

Though to this point we have been talking about mass screening, I would like to discuss one type of selective screening—that is, the screening of a selected group. Mental retardates, specifically, are more likely to have metabolic disorders than the general population. At first blush it would seem to be a waste of time, effort, and money to seek a diagnosis once mental retardation has already ensued, because in almost all instances therapy cannot reverse this damage. On the other hand, several other considerations point out the validity of testing mental retardates for metabolic disorders.

1) Establishing the cause of retardation enables better prognosticating and genetic counselling.
2) If therapy is started early with only mild retardation present, further deterioration might be prevented or slowed.
3) Identifying the inborn error allows a better determination of its incidence and therefore its possible worthiness for inclusion in mass screening programs.
4) Identifying the inborn error may give more insight into the various modes of clinical and/or biochemical presentation and therefore allow earlier recognition of future cases and determination of appropriate therapy.

There are almost as many screening tests as there are inborn errors of metabolism, but by selecting a few tests that will identify several different disorders, a workable screening panel can be established. The table depicts such a panel of tests established initially by R. B. Young (Department of Pediatrics, Medical College of Virginia) and continued presently by myself as a service to physicians in the State. In the present practice, fresh acidified urine of any patient with suspected mental retardation, or with a clinical complex to suggest an inborn error, is mailed to the Pediatric Metabolism Laboratory at the Medical College of Virginia. These tests are performed once weekly and the results mailed promptly to the referring physician. With more experience with these programs, it is expected that these tests will have to be modified (as has recently been found to be the case for the cyanide nitroprusside and C-TAB tests), and others added.

Summary

I have briefly outlined the PKU program in the State of Virginia and tried to demonstrate how this screening experience has disclosed the heterogeneity of the disease plus the need for an individualized approach to dietary control. I have applied the principles of mass screening to examine the feasibility of testing for other inborn errors and, on this basis, feel that galactosemia will soon next join with PKU. Mass screening for other aminoacidopathies will await more refined testing techniques and a definition of their incidence and mode of therapy. In the meantime, accumulated experience thus far mandates the establishment of specialized lab facilities and multidisciplinary teams; these will be necessary to effect optimal evaluation and treatment of patients found to be positive by these screening techniques.

References

Anderson VE, Siegel FS, Tellegen A, et al: Manual dexterity in phenylketonuric children. Percept Motor Skills 26: 827, 1968

Berman JL, Cunningham GC, Day RW, et al: Causes for high phenylayanine with normal tyrosine. Amer J Dis Child 117: 54, 1969

Berry HK, Wright S: Conference on treatment of phenylketonuria. J Pediat 70: 142, 1967

Clow C, Scriver CR, Davies E: Results of mass screening for hyperaminoacidemias in the newborn infant. Amer J Dis Child 117: 48, 1969

Davens E, et al: Statement on treatment of phenylketonuria. Pediatrics 35: 501, 1965

Hansen RG: Hereditary galactosemia. JAMA 208: 2077, 1969

Pathogenesis of Secondary Anemia*

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Introduction

As a simplification, it might be said that nutritional disturbances of short duration often cause a decrease in the serum or tissue concentration of a particular nutrient. If the disturbances last longer, they may next affect the bone marrow where rapid cell growth causes a high requirement for some nutrients. Since it is the erythrocyte precursors which grow most rapidly (Reizenstein et al, 1961; Reizenstein, 1962; Cea, Skårberg and Reizenstein, 1968), anemia is often an early malnutrition symptom. If nutritional disturbances last very long, severe changes in the body composition and weight loss occur (Kjellberg and Reizenstein, 1970; Kjellberg and Reizenstein, 1970).

There are a number of disease states, however, which are characterized by a decrease in the serum concentration of some nutrients, although no deficient intake is suspected. The classic example is the low serum iron in infection (Heilmeyer et al, 1958; Heilmeyer et al, 1960) which appears to be one contributory cause of anemia; another is hemolysis caused by extracorpuscular factors. In cancer, similar changes are found (Lockner, 1960); and if the inflammation or the tumor last long, severe weight loss can occur. It has been said of cancer patients that they "starve in the midst of plenty," but it is not known whether a decrease of appetite is enough to explain the nutritional disturbance or whether changes in the metabolism of nutrients occur as well.

Methods

The serum folic acid activity (SFAA) was measured with a bioassay method earlier described by Rama Rao and associates (1965). An intravenous injection was then made containing 15 μg folic acid per kg body weight and about 3 μc ^{59}Fe as ferrous citrate. Blood samples were obtained at intervals after the injection. Their radioactivities were measured in a Packard gamma spectrometer and their SFAA measured by the bioassay method. In this way the plasma elimination (PE) of folic acid and of ^{59}Fe could be determined. Later studies have been made of the PE of ^{198}Au as colloidal gold (approximately 8 μc, 0.1—7 mg gold, particle size 15–30 mμ), which estimates the phagocytic activity of the recticulo-endothelial system.

Results and Discussion

Metabolism of Iron and Folate

In the studies to be summarized here, some of which have already been published, the metabolism of some nutrients has been studied in 445 patients (Table I). The studies began when an attempt was made to use leukemias as examples of anemias of the non-megaloblastic type in a study of the serum folate in megaloblastic anemias (Hoogstraten, Baker and Reizenstein, 1963). It turned out that many of these patients had low folates. Later, similar findings were made in other forms of malignancy, correlated in part to the histology of the tumor (Fig 1) (Rama Rao et al, 1963; Rama Rao et al, 1965). Nothing was known of the pathogenesis of the low serum folate, but an increased cellular proliferation has been suggested (Mollin and Waters, 1968). Further studies demonstrated that an increased PE of folic acid apparently explains the low serum concentration (Einhorn and Reizenstein, 1966). Similarly, the hyposideremia seems to be explained by a rapid PE of iron (Heilmeyer et al, 1958; Heilmeyer et al, 1960; Lockner, 1960; Wiklund et al, in press).

Effect of Tumor Stage

In a group of patients with cancer of the uterine cervix—a form in which the stage of the tumor can be easily determined—an attempt was made to examine the relationship between the plasma elimination and the stage of the tumor. With the aid of a computer program developed for the purpose (Reizen-

* Secondary Anemias, XI. This work was supported by the Swedish Medical Research Council and the Cancer Foundation and partially performed in collaboration with G. Birke, J. Einhorn, N. Einhorn, A. Elman, B. Gheorghescu, B. Olhagen, S. O. Liljedahl and B. Wiklund. Article summarizes a guest lecture at MCV, Jan. 20, 1969.

stein and Zachrisson, 1968), significant correlations could be established (Fig 2) between the stage of the tumor, the iron and folate, and the anemia (Einhorn and Reizenstein, to be published). The more advanced the cancer is, the lower are the folates, and the more anemic is the patient. Similarly, a correlation between the "severity" of leukemia, the serum folate, and the anemia could be demonstrated (Fig 3). The more advanced the leukemia is, the lower are the folates, and the more anemic is the patient. In separate studies, it has been shown that neither in leukemia, preleukemia nor early myeloma (benign monoclonal gammopathy) is it a replacement of the erythroblasts in the bone marrow which causes anemia. There are often normal erythroblast numbers in the total body. Therefore, metabolic changes such as those discussed above may be one of the causes of the secondary anemia. Finally, patients with curable cancer of the uterine cervix were studied prior to and after treatment (Einhorn and Reizenstein, to be published), and the more successful the cure was, the more normal the plasma elimination became.

There are some indications that other nutrients are involved. The decrease in atheromatosis that can be demonstrated in cancer patients may suggest a disturbance in lipid metabolism (Table II).

Poor Appetite?

Is a decrease in appetite enough in those patients with malignancy to explain the pathological metabolism of some nutrients? This is conceivable, for an increase in PE of nutrients follows some deficiency states secondary to reduced intake (Mollin and Waters, 1968). To examine this question, the PE of folate was studied before and after the correction of a possible nutritional deficiency. Large quantities of folic acid were given, but the PE of folic acid was not normalized. These results suggest that the increased PE of iron and folic acid in patients with malignant disease is not secondary to a reduction in appetite alone, but that a disturbance in the PE of nutrients is a direct effect of the primary disease. The question now is how this effect is produced. Is it produced by tumors only?

Non-Malignant Tissue Damage

We know from the studies of Heilmeyer (Heilmeyer et al, 1958; Heilmeyer et al, 1960) and of many others that an accelerated PE of iron also occurs in infection. Here it results in a maldistribution of iron, which is deposited in the reticulo-endothelial cells and is thus unavailable for erythropoiesis. It could be shown (Elman and Reizenstein, 1963; Elman et al, 1964; Mollin and Hoffbrand, 1965; Elman et al, 1970) that the PE of folic acid was also increased in non-infectious inflammatory disease such as rheu-

TABLE I

Patients included in this study*

Diagnosis	Number of Patients
Leukemia	46
Malignant lymphoma	14
Cancer of the uterine cervix	57
Other forms of cancer	64
Inflammatory disease, ischemic necrosis, thyrotoxicosis	103
Operative trauma	21
Controls	140

* Many studies performed only in some of the patients.

December, 1968.

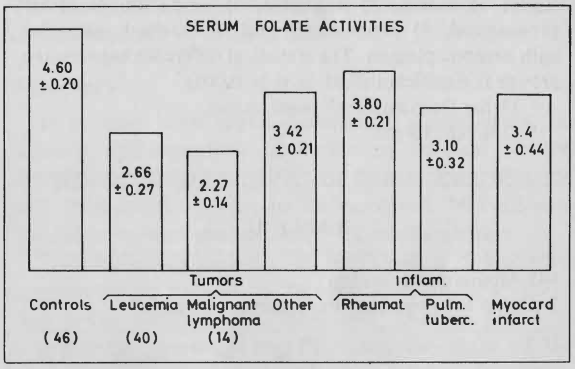

Fig 1—Serum folates in patients with different tumors and inflammations.

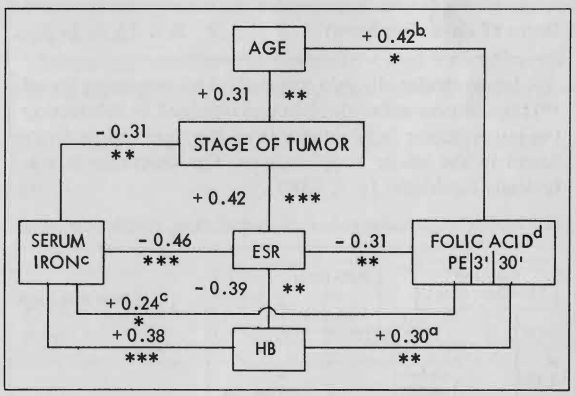

Fig 2—Secondary nutritional anemia in 78 patients with cancer, prior to treatment. Lines indicate correlations between the clinical observations (*—significant at the 95% level, ** at the 99.0 and *** at the 99.9); figures and signs indicate correlation coefficients. The more advanced the tumor, the lower the serum iron and folates, and the more severe the anemia. PE is folate plasma elimination rate. Concentrations are also indicated, 3 and 30 minutes after injection. a. Hemoglobin had the correlation +0.25 to folates at 15'. b. Same correlation coefficient found between age and folates at 3' and 30'. c. Serum iron was also correlated ($\gamma = +0.24$) to pre-load folates. d. Pre-load folates were correlated ($\gamma = 0.61$) to folates 15' after injection.

TABLE II

Atheromatosis at post mortem* in patients with malignant tumor. Means ± SE of mean.

	Cancer§	Other Patients‡
No. of patients	50	50
Mean age, years	61, 26 ± 1, 36	65, 54 ± 0, 49
Degree of atheromatosis† (1–6)	3, 28 ± 0, 25	4, 34 ± 0, 22

* Karlöf I, Reizenstein P: (1951) unpublished.

† A subjective classification of atheromatosis was used by several observers, who did not know the purpose of the study: 1) absent, 2) negligible, 3) moderate, 4) rather pronounced, 5) pronounced and 6) calcified, extensive with necrotic plaques. The statistical difference between the groups is significant ($0.01 > p > 0.001$).

‡ Dying from non-malignant causes.

§ Different forms.

TABLE III

^{131}I-Albumin distribution
Relation extravascular/intravascular albumin*

	M	M	Range	S.E.
Controls	5	1, 3	1, 1–1, 6	0, 07
Burns (7 days after burn)	9	3, 9	2, 4–12, 5	0, 31

* Intravascular albumin was studied by measuring blood ^{131}I; the extravascular albumin was obtained by subtracting the intravascular radioactivity from the total radioactivity found in the whole body counter. The difference is statistically significant ($p < 0.001$).

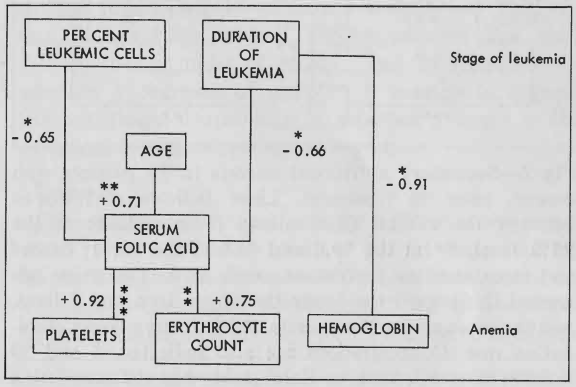

Fig 3—Secondary anemia in patients with chronic myelocytic leukemia. See legend for Fig 2. The longer the duration of the leukemia and the higher the proportion of leukemic cells, the lower the serum folic acid and the blood counts.

matoid arthritis (Fig 4), and that acceptable and significant correlations were found between the iron and folate turnovers. The longer the duration of the disease and the higher the erythrocyte sedimentation rate, ie, the worse the disease, the lower were the serum iron and cholesterol and the folate, and the more anemic the patients became. Similar findings could be demonstrated in inflammatory diseases of an infectious nature, such as pulmonary tuberculosis (Mollin and Waters, 1968; Elman et al, 1970). A similar decrease in the serum precipitable iodine, which was correlated to the decrease in albumin, has been demonstrated earlier in chronic inflammation and malignancy (Engström and Markardt, 1955), and of magnesium in burns (Broughton, Andersson and Boroden, 1968).

The finding of an increased PE of iron and folate in inflammatory as well as in neoplastic tissue damage did not suggest that the factors causing it could originate exclusively in tumors. For this reason we studied the effect of the damage of normal tissues of a nature other than inflammatory or neoplastic. We examined the effects of ischemia and of mechanical and thermal trauma.

The serum folate was shown to be low in patients with myocardial infarction (Elman et al, 1969) where we also observed low concentrations of serum iron. However, this has not yet been systematically studied. As for mechanical trauma, patients subjected to operations with minimal bleeding (inguinal hernia) were studied prior to and the day after operation. In every single patient the PE of iron was more rapid after operation. However, these patients were subjected to trauma and to at least some unavoidable bleeding, and it was uncertain which of these two factors caused the increased PE of iron. For this reason, the effect

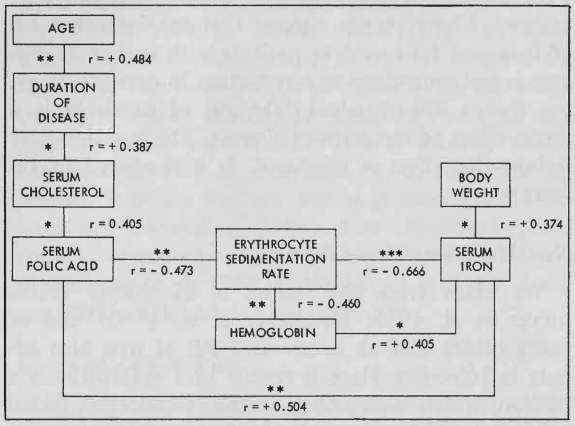

Fig 4—Secondary anemia in rheumatoid arthritis. See legend for Fig 2. The longer the duration of the disease, and the more severe the disease as indicated by the erythrocyte sedimentation, the lower the folates, iron, and cholesterol, and the more severe the anemia.

of trauma without bleeding (operations of the lateral meniscus in a bloodless field) and that of bleeding without trauma (phlebotomy 100–150 ml) were studied. The result suggested that it is the trauma which is responsible for the increased PE of iron (Åsén et al, 1969) and not the bleeding.

In the group with thermal injury, only the PE of albumin has been studied so far (Birke et al, 1968). An increased PE with a maldistribution of albumin was found even after small burns (Table III).

Role of Factors Released

A similarly increased PE of albumin can be demonstrated after other forms of trauma; and it has also been shown that the serum concentration of albumin is reduced in tumor patients (Reizenstein, 1970).

The findings described have been summarized in Table IV. If an increased PE and a decreased serum concentration of every nutrient had been found in all the patients with tissue damage of a malignant inflammatory, ischemic or traumatic nature, then it would be quite likely that the effect of tissue damage was to cause a rapid plasma elimination and a decreased serum concentration, ie, a maldistribution of some erythropoietic factors. Some substances like albumin and cholesterol not involved in erythropoiesis, but in the general nutritional condition of the patient, appear to be similarly affected. In fact, the metabolism of proteins with a molecular weight around 70,000 like albumin and transferrin might even be intimately related to that of folate, cholesterol, iodine, iron and magnesium.

Almost nothing is known about what causes the accelerated plasma elimination. Cortisone, thyroxine and serotonin apparently do not cause an increased PE (Åsén et al, 1969; Birke et al, 1968). The effect appears to be related to increased capillary permeability and to the reticulo-endothelial activity. In postoperative patients, the latter is significantly increased. Both the synthesis and breakdown of fibrinogen—possibly in reticuloendothelial cells—are increased after operation (Davies, Liljedahl and Reizenstein, 1970) and this could explain the correlations found between the PE rates and the erythrocyte sedimentation rates.

The reticulo-endothelial system could also be responsible for the immediate post-traumatic hemolysis even of red cells formed before the trauma. Whether it is stimulated by antigenically active factors, liberated by tumors or damaged tissue, we do not know. The immuno-depressant cytoxan "normalizes" the PE of iron, but not of radio-gold.

Summary

In patients with tissue damage of a malignant, infectious, inflammatory, ischemic or surgical nature, the plasma elimination (PE) of folates, iron, albumin and cholesterol appears to be increased. Not all substances have been studied in all diagnostic groups.

The secondary anemia in myeloma and leukemia appears to be caused by factors other than a reduction in the number of erythroblasts. There is generally a correlation between the PE rates, the stage of the tumor or inflammation, and the anemia. It is suggested that the mechanism demonstrated contributes to the development of the anemia and possibly to the weight loss.

Increased capillary permeability and reticulo-endothelial activity could possibly explain PE and break-

TABLE IV

Rapid PE or low concentration (both marked +) of nutrients in serum in patients with ischemic, inflammatory, malignant or traumatic tissue damage. Brackets indicate results from literature.

	Fe*, conc.	Fe, PE	PGA, conc.	PGA, PE	CHOL conc.	ALB., PE	E.S.R.
Cardiac infarction	+		+		+		
Inflam.: Rheum. arthrit.	+	+	+	+	+	+	+
Pulm. TBC	+	+	+	(+)		(+)	+
Tumor: Leuk.			+	(+)	(+)		
Lymphoma			+	(+)	(+)		
Cancer		+	+	+	+		(+)
Trauma	+	+				+	(+)

* Abbreviations indicate iron, folate, cholesterol and albumin.

down of small proteins and substances carried by them, as well as phagocytosis and lysis of some red cells.

References

Åsén P, Birke G, Liljedahl SO, et al: Studies of trauma III. Plasma iron elimination rate. Acta Chir Scand 135: 257, 1969

Birke G, Liljedahl SO, Plantin LO, et al: Studies on burns IX. The distribution and losses through the wound of ^{131}I-albumin measured by whole-body counting. Acta Chir Scand 133: 27, 1968

Broughton A, Andersson I, Boroden C: Magnesium deficiency in burns. Lancet 2: 1156, 1968

Cea R, Skårberg KO, Reizenstein P: Polycytemia Vera—total number of erythroblasts and their generation times. Int Soc of Hematology, XII Congress, New York, 1968

Davies JWL, Liljedahl SO, Reizenstein P: Fibrinogen metabolism following injury and its surgical treatment. Injury 1: 178, 1970

Einhorn J, Reizenstein P: Metabolic studies on folic acid in malignancy. Cancer Res 26: 340, 1966

Einhorn N, Reizenstein P: Folic acid in early cervical cancer. (To be published)

Elman A, Reizenstein P: Serum folic acid in patients without expected folic acid deficiency. Swedish Physicians National Conference, Stockholm, 1963, p 173

Elman A, Reizenstein P, Hanngren Å, et al: Possible reasons for disturbed folic acid metabolism. Swedish Physicians National Conference, 1964, p 210

Elman A, Einhorn J, Olhagen B, et al: Metabolic studies of folic acid in non-malignant diseases. Acta Med Scand 187: 347, 1970

Engström W, Markardt B: The effects of serious illness and surgical stress on the circulating thyroid hormone. J Clin Endocr 8: 953, 1955

Heilmeyer L, Keiderling W, Bilger R, et al: Anaemia refractoria sideroblastica. Folia Haemat 2: 49, 1958

Heilmeyer L, Keiderling W, Merner H, et al: Anaemia refractoria sideroblastica. Acta Haemat 23: 1, 1960

Hoogstraten B, Baker H, Reizenstein P: Correlation between folic acid activity and response to antifolate therapy. Blood 18: 787, 1961

Kjellberg J, Reizenstein P: Body composition in obesity. Acta Med Scand 188: 161, 1970

Kjellberg J, Reizenstein P: Effect of starvation on body composition. Acta Med Scand 188: 171, 1970

Lockner DL: Klinische Untersuchungen zur Krebsanämie. Acta Haematologica 24: 196, 1960

Mollin DL, Hoffbrand AV: Series Haemat 3: 1, 1965

Mollin DH, Waters AH: Nutritional megaloblastic anaemias. *In* Occurrence, Causes and Prevention of Nutritional Anaemias. Stockholm, Almquist & Wiksell, 1968, p 121

Rama Rao PB, Lagerlöf B, Einhorn J, et al: Low serum folic acid in patients with malignant disease. Lancet 1: 1192, 1963

Rama Rao PB, Lagerlöf B, Einhorn J, et al: Folic acid activity in leukemia and cancer. Cancer Res 25: 221, 1965

Reizenstein P, Cronkite EP, Bond VP, et al: Implications of cell flow between morphologic compartments on H^3 Thymidine labeling index (LI) variations in human bone marrow. Fed Proc 20: 68, 1961

Reizenstein P: Implications of the total erythrocyte production in man on erythroblaste generation times. Acta Med Scand 172: 685, 1962

Reizenstein P, Zackrisson L: Mapping of correlation between clinical variables. International Congress of Medicine, Warsaw, Abstracts, 1968, p 168

Reizenstein P: Leukemia and nutrition. (Review) Nord Med 83: 74, 1970

Wiklund B, Gheorghescu B, Liljedahl SO, et al: Secondary anemias V. Reticulo-endothelial activity and some metabolic changes related to trauma. Adv in Exp Med and Biol, In press

SAMA-AΩA Student Honors Day: Abstracts of Scientific Presentations

Medical College of Virginia, May 1970

A Technique for the Measurement of Reagenic Antibody: Technical Development and Future Studies

PETER F. HOFFMAN (M–70)

The purpose of this study was to establish in our laboratory a method for the measurement of reagenic antibody, now known as Immunoglobulin E (IgE). Immunoglobulin E specifically contains within it the antibody moiety that is responsible for reactions of the immediate hypersensitivity type in man. The reaction of IgE with specific allergens has been shown to lead to the release of histamine *in vitro*. *In vivo* it appears to be the immunoprotein involved in extrinsic asthma, hayfever, atopic dermatitis and anaphylactoid drug reactions. Because the normal level of IgE in the serum is only approximately 250 ± 100 nanograms/ml (ng/ml), more conventional techniques for measuring immunoglobulin levels such as the single radial diffusion method are not sensitive enough to determine the concentration of IgE. In order to quantitatively detect IgE a modification of the solid phase bromacetyl cellulose radioimmunoassay technique (Mann, et al. J. Imm. 102: 618, 1969) was undertaken. The principle of the technique is the inhibition of the binding of a trace (less than 0.1 ng) of "hot" or I^{125}-labelled IgE to an insoluble polymer of anti-IgE coupled with bromacetyl cellulose (BAC:anti-IgE) by unlabelled or "cold" IgE. By first establishing a standard curve it is possible to detect the presence of IgE down to approximately 1 ng/ml. The methodology necessary to instrument this procedure in our laboratory will be discussed. Elevated levels, up to 100 times normal, have been reported in patients with asthma, atopic dermatitis, and anaphylactoid drug reactions. Unfortunately there has been little clinical correlation between elevated IgE levels and specific symptomatology. Over the next year we will be attempting to measure both total IgE levels as well as specific IgE antibody levels in patients with drug reactions, particularly penicillin reactions and we will attempt to better define the relationship between IgE and adverse anaphylactoid drug reactions. Hopefully this will lead to a more precise ability to prejudge whether or not a particular patient will or will not experience a potentially devasting reaction when given a drug such as penicillin.

Preceptor: W. Kenneth Blaylock. *Division of Dermatology, Medical College of Virginia.*

Suppression of Homograft Immunity with Thiamphenicol

J. D. LINEHAN (M–70)

Thiamphenicol, the methylsulfonyl analogue of chloramphenicol, is believed to suppress newly induced antibody production by inhibiting messenger—RNA at the cellular level. Accordingly, thiamphenicol was evaluated in prolonging canine renal homograft and canine cardiac homograft survivals. METHODS—18 dogs underwent bilateral nephrectomy and renal homotransplantation, receiving thiamphenicol as sole immunosuppressant. 14 control dogs—same operation but no immunosuppression. 8 dogs underwent orthotopic cardiac homotransplantation, receiving thiamphenicol immunosuppression (prednisone added for acute rejection episodes). 27 control dogs from a previous series underwent the same operation but received no immunosuppression. Rejection was monitored by daily blood urea nitrogen (BUN) and serum creatine (S-Cr) determinations in renal homografts and daily electrocardiograms in cardiac homografts. RESULTS—Renal—Median survival of dogs receiving thiamphenicol was *17 days* (range 8–49 days), significantly greater ($p < .01$) than control group median survival of *9 days* (range 7–14 days). Only 6–18 dogs

receiving thiamphenicol died primarily of rejection; other dogs died of infection and pneumonia. Cardiac—Median survival of dogs receiving thiamphenicol was *29 days* (range 5–250 days) versus *7 days* median control survival (range 4–21 days). One dog receiving thiamphenicol is currently alive 250 days post transplant. Histopathologic changes in the renal and cardiac homografts will be presented, as well as side effects of thiamphenicol therapy. SUMMARY—Thiamphenicol is a potent immunosuppressive agent, producing significantly prolonged canine renal and cardiac homograft survival quite similar to that produced by azathioprine and 6-mercaptopurine, which have different mechanisms of action. Because of its immunosuppressive properties, thiamphenicol may be a useful adjunct in clinical immunosuppression.

Preceptors: David M. Hume and H. M. Lee. *Department of Surgery, Medical College of Virginia.*

Blood Volume Regulation Following Cardiac Transplantation*

MARC D. THAMES (M–70)

The changes in blood volume and the peripheral plasma renin responses to hemorrhage were studied in dogs following cardiac transplantation. Each dog was anesthetized with pentobarbital, blood volume was determined by dye dilution technique (Evans blue), and control samples for plasma renin were obtained. Then the animal was bled 15 ml/kg and samples for plasma renin were drawn 20 min. and 30 min. after hemorrhage and 15 min. after reinfusion of the blood. The blood volume of dogs following cardiac transplantation before reinnervation of the heart was significantly greater than that of normal dogs. Plasma renin of normal dogs rose to 340% of control following hemorrhage, while that of animals with cardiac transplantation rose to only 180% of control value. This difference was significant. To determine if this difference was due solely to the expanded blood volume found in the denervated animals, 6 normal dogs were transfused with 25 ml/kg of whole blood the day prior to study. The renin response to hemorrhage in these dogs was similar to that of dogs with cardiac transplantation. However, 4 dogs with cardiac transplantation diuresed with i.v. ethacrynic acid 1 mg/kg the day prior to study so that their blood volumes were near that of normal dogs showed no increase in plasma renin following hemorrhage. These results indicate that dogs with cardiac denervation have increased blood volumes and show a subnormal renin response to hemorrhage which cannot be attributed entirely to expanded blood volume. These data are consistent with the view that there are afferent receptors in the heart which participate in the reflex regulation of blood volume and that this mechanism is interrupted by denervation of the heart subsequent to cardiac transplantation.

Preceptor: Hermes A. Kontos. *Department of Medicine, Division of Cardiovascular Disease, Medical College of Virginia.*

* Abstract submitted for publication to *American Journal of Physiology.*

The Roentgenographic Diagnosis of Aneurysms of the Superior Mesenteric Artery with Two Case Reports

PARHAM R. FOX (M–71)

Superior mesenteric artery aneurysms are rare. The majority of the cases are diagnosed at autopsy, and the incidence is approximately one in 12,000 consecutive unselective autopsies. In a review of the world literature, De Bakey and Cooley found a total of 63 cases of aneurysms of the superior mesenteric artery reported up to 1953, and there have been at least 8 additional cases reported to date. The majority of these aneurysms are mycotic and occur as a complication of bacterial endocarditis. The clinical picture of aneurysms of the superior mesenteric artery is not characteristic. The occurrence of epigastric pain associated with an epigastric mass in a patient with bacterial endocarditis should arouse suspicion of a mycotic aneurysm of the superior mesenteric artery. On physical examination, expansile pulsation and a systolic bruit are additional evidence for this diagnosis. The ability to displace the pulsatile mass laterally is also very important. Epigastric pain associated with back pain which may be clinically indistinguishable from cholecystitis or pancreatitis has also been described. The first patient, a 70 year old white male, presented with obstructive jaundice, an unusual clinical manifestation of aneurysms of the superior mesenteric artery, occurring more frequently with aneurysms of the hepatic and celiac artery. Gastrointestinal hemorrhage has been described as another symptom of hepatic, celiac and pancreaticoduodenal artery aneurysms, but not before with superior mesenteric artery aneurysms. Roentgenograms

of the abdomen may only reveal a soft tissue mass in the epigastrium. This is a non-specific finding and will not permit the diagnosis of aneurysm of the superior mesenteric artery. However, if there is calcification in the wall of the aneurysm, the location and characteristics of this calcification are very important. The calcification is curvilinear and is usually located in the midline or just slightly lateral to the midline, approximately one-half lumbar vertebral body width anterior to the bodies of the upper lumbar vertebra. In our cases, and in the published cases with roentgenographic illustrations, there is a posterior defect in the continuity of the circumferential calcification shown in the lateral projection which represents the origin of the superior mesenteric artery or its major proximal branches. This is a distinctive finding and permits a presumptive diagnosis. However, aneurysms of the celiac artery which are less common, could theoretically have similar plain film findings. The definitive diagnosis can be made with angiography, preferably with selective visceral angiography. It is important not only to make the diagnosis of aneurysm, but also to demonstrate whether or not occlusion of the superior mesenteric artery has occurred and whether or not collateral circulation is present. Selective angiography also permits the demonstration of the relationship of the celiac axis to the aneurysm and patency of the superior mesenteric vein. In the second case, a 72 year old Negro female, the celiac axis was displaced superiorly and draped anteriorly over the aneurysm. In view of the high mortality rate associated with this lesion, preoperative mapping of the vascular tree is very important in order to plan surgical correction.

Preceptor: William A. Weidner. *Department of Radiology, Medical College of Virginia.*

Studies on the Clearance of Circulating Leucocytic Pyrogen*

M. TENENBAUM (M–71) AND
D. LORBER (M–72)

Fever is believed to be caused by action on the hypothalamus of a protein pyrogen released by leucocytes. The magnitude of the fever appears to be dependent on the amount of pyrogen circulating in the blood. The present studies were designed to determine the site of removal of leucocytic pyrogen (L. P.) from the circulation. Rabbits were given endotoxin fevers and their urine collected during the time that they were febrile. As much as 200 ml urine from febrile rabbits was nonpyrogenic when injected intravenously. Crude rabbit leucocytic pyrogen was prepared from rabbit peritoneal exudate cells which were incubated for 8–12 hours in phosphate buffed saline solution. When urine from afebrile rabbits was incubated with crude leucocytic pyrogen, no significant decrease of pyrogen activity was observed. Similarly no decrease in pyrogenic activity was observed after incubating L. P. with rabbit plasma. However, when rabbit livers were perfused with fluid containing leucocytic pyrogen, all pyrogen disappeared within 15 to 45 minutes from the perfusate in 7 out of 9 perfusion experiments. The BSP test after perfusion was normal in those 7 perfusions. In the two experiments in which some L. P. remained after 45 minutes, the BSP tests indicated liver damage. The data indicate that leucocytic pyrogen is not directly excreted in the urine or inactivated in the plasma or urine. The liver appears to be the site of destruction of circulating pyrogen.

Preceptor: George W. Gander, *Department of Pathology, Medical College of Virginia.*

* Abstract submitted for publication to *Proceedings of the Society for Experimental Biology & Medicine.*

Study of the Combined Effect of Pyran Copolymer with Actinomycin D or Cytoxan on Some Mouse Tumors

STEVEN H. GROSSMAN (M–73)

Through the use of Pyran Copolymer, a reticuloendothelial system stimulant, some cancers have been retarded. Many other drugs have been used effectively as carcinotoxic agents. This series of experiments has been undertaken to explore the possible synergistic effects of combining these drugs with Pyran, representing increased host resistance. Three tumor systems were used: Exp. I. solid Ehrlich tumor placed sub-cutaneously; Exp. II. Ehrlich ascites tumor; and Exp. III. Friend Virus Leukemia. Pyran causes depressed function of the RES for seven to eight days beginning at 24 hours and stimulation thereafter. Experiment I used six groups of Swiss mice: (1) Tumor control (2) Tumor plus Cytoxan (3) Tumor plus Pyran (RES stimulated) (4) Tumor plus stimulation plus Cytoxan (5) Tumor plus RES depressed (6) Tumor plus RES depressed plus Cytoxan. Cytoxan (cyclophosphamide) therapy produced 51.9% tumor inhibition, Pyran therapy produced 80.5% tumor inhibition when the tumor was received during RES stimulation, and 85.8% when depressed.

Groups four and six produced weaker inhibition (79.1% and 70.2%, respectively) than with Pyran alone. Colloidal carbon clearances had shorter T½'s than in controls or in untreated tumors. Individual organ weights were greater in all cases compared to controls but not necessarily to untreated tumor mice. Experiment II utilized cummulative survival time for evaluation. Mean survival time was not increased significantly in relation to the total duration of the study, in any group. Experiment III used individual organ weights in evaluating the combined therapy against the Friend Virus Leukemia. Spleens, livers, and lungs were generally enlarged and no tumor inhibition was demonstrated. These three experiments have failed to demonstrate an additive antitumor effect as postulated, however, this methodology has enabled further studies to demonstrate positive results using methotrexate, 5-flurouracil, hydroxyurea, and other drugs in conjunction with Pryan.

Preceptor: William Regelson. *Department of Medicine, Medical College of Virginia.*

Trypanosome Infection and Tumor Growth

GARY HOFFMAN (M–72)

The effects of *Trypanosoma duttoni* and *T. lewisi* infections were studied in mice bearing either Friend Virus Leukemia or Leukemia L-1210 and appropriate controls. Both species of trypanosomes are non-pathogenic and have been previously shown to be potent stimuli of the reticuloendothelial system. Mice were either immunized to trypanosome infection, or infected with trypanosomes at the time of tumor inoculation. In neither case was diminished tumor growth noted in reference to tumored controls.

Preceptor: William Regelson. *Division of Medical Oncology, Medical College of Virginia.*

Plasma-Pressor Activity in Metabolic Acidosis

F. J. MARTORANO (M–71)

Arterial blood from twenty-one patients was analyzed for pO2, pCO2, pH, and plasma-pressor activity (PPA) to assess the effect of hypoxemia, hypercapnia, and acidosis on catecholamine levels in the plasma. PPA was measured using a semimicro (0.2 ml.) bioassay utilizing an isolated rabbit ear artery. Pressor response was expressed in norepinephrine equivalents (nanograms per ml.). Specificity studies in our laboratory indicated that PPA measured in this bioassay reflects primarily the plasma concentration of active catecholamines. Fifteen patients had an arterial pH greater than 7.3 associated with a PPA of less than 2 ng/ml. The remaining six patients were in metabolic acidosis with pH ranging from 7.08 to 7.28 and demonstrated PPA levels from 2.5 to 25 ng/ml. There was no direct correlation between arterial pO2 and pCO2 with PPA. In five of the six acidotic patients, further monitoring during medical management showed the PPA to vary inversely with arterial pH. This work confirms previous animal studies by other investigators which demonstrated acidosis to be an important primary stimulus for catecholamine release rather than hypoxemia or hypercapnia *per se*.

Preceptor: Reuben H. Young. *Department of Pediatrics, Medical College of Virginia.*

Specificity of Ribonuclease Activity as Related to Homograft Rejection

DAVID WALDMAN (M–72)

Many methods to detect or confirm homograft rejection have been devised utilizing change in hemodynamics, immunological reactions, enzymatic change, and biopsies. Utilization of many different approaches as well as poor sensitivity and specificity denotes the complexity of the rejection mechanism. Jolley and others have suggested studying rejection processes with an enzymatic approach and showed the change in ribonuclease activity relating to skin graft rejection. This project was undertaken to study the correlation of ribonuclease activity with renal homograft rejection with regard to its specificity and

sensitivity and possible use of this approach to help elucidate the rejection mechanism. METHOD—Mongrel dogs weighing 10 to 15 kgm were used as an experimental animal and kidney transplants were done to the pelvic vessels according to the established technique in this lab. Blood urea nitrogen and creatinine levels were followed in all animals measured by the autoanalyzer. Dogs were autopsied and histology sections made of the kidney, lymph node, liver and lungs. Serum ribonuclease activity was assayed by the Roth method at pH 5.8 and pH 7.8. Control samples were obtained on all animals prior to experimentation and all samples were measured in triplicate ribonuclease activity at intervals during the experiments. Animals were grouped into four categories: (1) autotransplants, (2) hydronephrosis, (3) renal infarct and (4) homotransplant. Homotransplant was subdivided into two groups, (1) with uremia, having homotransplanted kidney as sole kidney, (2) without uremia, having one of his own kidneys left in to exclude uremia as a variable factor. RESULT —We have defined one unit of RNAse activity as a change of .001 O. D. over 30 minutes at 260 mu. Using this scale we have established a range of 625–710 units at pH 5.8 and 710–810 units at pH 7.8 as a normal control value in mongrel dog population. Our preliminary study showed no significant change in ribonuclease activity relating to sex and there were no significant diurnal variations or daily fluctuations. Immunosuppressive therapy with imuran and prednisone produced very slight but general decrease in activity at both pH 7.8 and pH 5.8. Homotransplant group showed a significant increase in ribonuclease activity at pH 7.8 while showing no change at pH 5.8. Absence of uremia did not modify this increase of ribonuclease activity. Uremic homotransplant group ranged to 1150–1700 units at 7.8 pH and nonuremic group 1150–2300 units at pH 7.8. Both hydronephrosis and renal infarct group showed slight increase in activity immediately after surgery at both pH 7.8 and pH 5.8. However they decreased to the normal control levels by the third postoperative day. This initial rise never reached the level of the homotransplant group increase. SUMMARY—There is a measurable increase in the serum ribonuclease activity at pH 7.8 in kidney homotransplant dogs regardless of the presence or absence of uremia. This seems to be related to homograft rejection itself rather than nonspecific kidney damage. Autotransplantation, renal infarct, or hydronephrosis failed to show any change in the ribonuclease activity. This result may suggest use of ribonuclease activity as detection or confirmation test of renal homograft rejection and also one approach to the elucidation of the rejection mechanism.

Preceptor: H. M. Lee. *Department of Surgery, Medical College of Virginia.*

An Increase in Brain Serotonin in Experimental Porphyria

WILLIAM R. REAMY (M–72)

Acute intermittent porphyria is a rare inherited disease. Biochemically it is characterized by an increase in the urinary excretion of δ-aminolevulinic acid (ALA) and porphobilinogen. The primary molecular lesion is an elevation in hepatic ALA synthetase (Tschudy *et al.*, 1965, Proc. Nat. Acad. Sci. *53,* 841), the rate-limiting enzyme in heme biosynthesis (Granick and Urata, 1963, J. Biol. Chem. *238,* 821). Clinically, acute intermittent porphyria is manifested by neurological disorders and is sometimes accompanied by depression, confusion, and visual hallucination (Wetterberg, 1967, *A Neuropsychiatric and Genetical Investigation of Acute Intermittent Porphyria,* Svenska Bokforlaget, Stockholm). The relationship between the aberration in heme synthesis in the liver and the nervous system is enigmatic. ALA synthetase can be induced in embryonic chick liver in vitro by a variety of compounds including barbiturates, collidines, and steroids (Granick, 1966, J. Biol. Chem. *241,* 1359). 3, 5-dicarbethoxy-1, 4-dihydrocollidine (DDC) is a potent inducer of ALA synthetase in embryonic chick liver in vivo (Simons and Boell, 1967, Am. Zool. *7,* 48). At thirteen days of development, when the enzyme is normally not yet present in detectable amount, DDC produces a massive increase in the enzyme—forty times the adult value. The production of experimental porphyria provides a useful approach to the study of the biochemical aspects of this disease. Experimental porphyria was induced in 13 day chick embryos by DDC and the concentration of serotonin measured in different regions of the brain by the fluorometric method of Snyder *et al.* (1965, Biochem. Pharmac. *14,* 831). The concentration of serotonin in the cerebral hemispheres of both normal and treated animals is less than that in the remainder of the brain. A significant increase in the concentration of serotonin occurs in porphyric animals in the noncerebral portion of the brain but not in the hemispheres.

A growing body of evidence implicates disturbances in serotonin metabolism in mental disorders. The elevation of brain serotonin accompanying the induction of hepatic ALA synthetase may be a concomitant of faulty transmission in serotonergic neurons, thereby eliciting through neurochemical events as yet unknown the neurologic symptoms which occur in acute intermittent porphyria. The induction of ALA synthetase in the liver involves genetic regulatory mechanisms (Granick, 1966). Heme apparently acts as a corepressor of the synthesis of messenger RNA

for ALA synthetase. The inducer competes with heme for the hypothetical repressor and renders it inactive, thereby allowing the synthesis of messenger RNA to proceed. Whether the increase in brain serotonin is a consequence of enhanced heme synthesis or whether it is an independent effect of DDC remains to be seen.

Preceptor: Jarid A. Simons. *Department of Biology, College of William and Mary.*

Inhibition of the Lupus Erythematosus Cell Phenomenon in Uremia

HAROLD L. REKATE (M–70)

Immunologic reactions may be deranged in uremic patients. We present clinical and laboratory evidence indicating that the L. E. cell test may be suppressed in such patients. Two patients at the Medical College of Virginia Hospitals have shown inhibition of the L. E. cell test due to their uremia. The first of these, T. Y., a 21-year-old male, was admitted for peritoneal dialysis and was found to be uremic with an elevated BUN and serum K of 9.61. The presumptive diagnosis was subacute glomerulonephritis. An L. E. cell test on admission was negative. After dialysis his BUN fell to 64 and a repeat L. E. cell preparation was found to be positive. Autopsy revealed evidence of lupus nephritis. The second patient, G. S., was a 36-year-old female known to have systemic lupus erythematosus with progressive renal insufficiency. On admission her BUN was 219 mg% and the L. E. cell test was negative. Following peritoneal dialysis her BUN fell to 68 mg% and a positive L. E. cell test was obtained. The mechanism of inhibition has been studied with tests designed to distinguish sensitization and phagocytosis phases of the L. E. cell phenomenon. There are two stages in the formation of an L. E. cell, and these can be separated in the laboratory. The first phase is the sensitization phase which is presumably an antigen-antibody reaction between a 7S globulin (the L. E. factor) and nucleoprotein. The second phase is the phagocytosis of the sensitized nucleoprotein, resulting in formation of the L. E. cell. In the first phase mouse liver nuclei are sensitized with a known positive L. E. serum to form "loose bodies" which correspond with hematoxylin bodies. The formation of these "loose bodies" is inhibited by the presence of uremic serum. The "loose bodies" obtained from stage 1 are then presented to competent phagocytes and L. E. Cells are formed (stage 2). The second stage is not inhibited by uremic serum. Fluorescent antibody studies (determinations of anti-nuclear factors) indicate that urea itself is responsible for the inhibition. Conclusions: (1) the L. E. cell test may be suppressed in patients with uremia. (2) This suppression has been diagnostically confusing. (3) Inhibition is relieved by peritoneal dialysis. (4) Inhibition is attributable to a dialyzable serum factor. (5) Inhibition affects the primary reaction of nucleoprotein with L. E. globulin. (6) The dialyzable factor is urea.

Assessment of the Role of Pancreatoduodenectomy in the Treatment of Chronic Relapsing Pancreatitis—Analysis of 16 Cases

DAVID E. MULLINS (M–70)

A review of 35 pancreatoduodenectomies (Whipple procedure) performed at McGuire V. A. Hospital between 1961–68, showed that 16 were done for chronic relapsing pancreatitis and 19 for carcinoma of either the pancreas, ampulla of Vater, or common bile duct. Because of continuing interest in the assessment of the results of surgery for chronic relapsing pancreatitis, these 16 cases were reviewed to evaluate the pre- and post-operative clinical course and to further evaluate the role of the Whipple procedure in the surgical management of chronic pancreatitis. Pre-operative evaluation included: history of pain, alcoholism, narcotic addiction, diabetes, and jaundice, and the presence of biliary tract disease, and pancreatic calcification and cysts. A history of alcoholism was present in 14 of the 16 patients and diabetes and pancreatic calcification was found in 10 patients. Seven patients had undergone 21 previous surgical procedure, prior to the Whipple operation, for the management of their pancreatitis, ie., biliary tract surgery, sphincterotomy, caudal pancreatic drainage, and vagotomy and antrectomy with Billroth II anastomosis. These operations failed to control the patient's recurrent pancreatitis if they continued to consume alcohol or if there was an enlarged, indurated mass in the head of the pancreas at the time of surgery. Post-operatively, three patients developed mild diabetes not present before surgery, 13 required supplemental oral pancreatic enzyme replacement because of weight loss and mild steatorrhea, three became Vitamin A deficient, and two developed osteomalacia. There was one hospital operative death from acute hemorrhagic pancreatitis of the remaining pancreas. Two patients died three and one-half years post-operatively from liver failure

and its sequellae secondary to continued alcohol consumption. The 13 surviving patients have to date demonstrated no further clinical progression of their pancreatitis. *Conclusions*: If the patient has an enlarged, indurated mass in the head of the pancreas at the time of surgery, the Whipple operation has adequately controlled further progression of their pancreatitis. However, only those patients who have a reasonable chance of eliminating alcohol from their diet and who are able to care for their pancreatic endocrine and exocrine deficiencies are candidates for the Whipple operation for control of their symptoms of chronic pancreatitis.

Abstracts of Theses for Graduate Degrees

Medical College of Virginia, June 1970

Radioimmunoassay of HGH and ACTH in Surgically Stressed Patients

JAMES CARRINGTON ROSE, M.S.
Department of Physiology

The development of a specific, highly sensitive radioimmunoassay (RIA) for adrencorticotrophic hormone (ACTH) is described. With this assay it is possible to measure normal plasma concentrations of ACTH without first extracting the peptide from the plasma. The range of plasma ACTH of normal subjects with this assay is 0 to 35 pg/ml when determined between 9 a.m. and 12 noon. High concentrations of plasma ACTH are associated with Cushing's disease, after bilateral adrenalectomy and the ectopic ACTH syndrome. Low concentrations are associated with treatment with dexamethasone and hypercortisolemia caused by an adrenal tumor.

A study of the human growth hormone (HGH) and ACTH response to surgery has been done. The plasma levels of both ACTH and HGH increase in response to surgical stress under general anesthesia. The increase in HGH concentration could not be attributed to hypoglycemia since blood glucose levels which were also monitored remained elevated. No significant increase in either hormone is seen following surgical stress under spinal anesthesia. This suggests that spinal anesthesia is capable of blocking the response of the pituitary to surgical stress. The preoperative medications used and the induction of anesthesia exhibit no significant effect on the plasma concentration of either hormone. The remarkable similarities observed in the increase in secretion of HGH and ACTH to surgical stress under general anesthesia suggest a similarity of pathways involved in this response. Also it appears that the stress of major surgery is capable of diminishing the effect of the glucose dependent negative feed-back mechanism involved with HGH secretion.

Tongue Rolling and Tongue Folding in an American Caucasian Population

MARTHA JANE RUEBUSH, M.S.
Department of Genetics

A sample of 1040 Caucasian students at the Health Sciences Division and the Academic Division of Virginia Commonwealth University were classified by visual observation according to the extent, if any, to which the tip of the tongue could be turned up without aid of teeth or lips and by whether or not the tongue could be rolled. Individuals varied in the extent to which they could turn up the tip of the tongue from not at all to folding the organ flat upon itself. The abilities to roll and to fold the tongue were independent, but only if folding was classified as folding the tip of the tongue flat upon the organ. Proportionally more males (75.81%) than females (67.49%) in the sample could roll the margins of the tongue, but there was no difference between the frequencies of males (4.61%) and females (3.47%) who could fold the tongue flat. The frequencies of rolling and of folding in the sample were in close agreement with the frequencies in other Caucasian populations and differed to various degrees from the frequencies in other races. Comparisons of the frequencies of rolling and of folding in some African tribes, an American Negro population, and in Caucasian populations, including the present study, suggested that the different frequencies in closely related human populations are more likely to be results of genetic drift in isolates than of natural selection.

Study of the Toxic, Pyrogenic, and Cytopathogenic Properties of Lipopolysaccharide of *Salmonella Abortus Equi*

BRENDA LEE WRIGHT, M.S.
Department of Pathology

A study was made of the toxic, pyrogenic, and cytopathogenic properties of the lipopolysaccharide of *Salmonella abortus equi* endotoxin. The *S. abortus equi* was a commercially obtained Westphal phenolic extracted lipopolysaccharide (LPS). The LPS was treated according to the method of Neter, Westphal, Lüderitz, Gorzynski, and Eichenberger (1956). The preparations, NaOH/6 min., NaOH/60 min., PBS/6 min. (phosphate buffered saline), and PBS/60 min. were tested in three systems. Toxicity of the preparations was determined in mice, pyrogenicity in rabbits, and cytopathogenicity in Wi-38 cell culture. By the imposed treatment, the ability to exert a cytotoxic effect is retained while toxicity for mice is greatly reduced and pyrogenicity for rabbits reduced to a lesser degree. The rabbit pyrogen test was shown to be 100,000 times more sensitive than the mouse lethality system and the cell culture system 10 times as sensitive as the rabbit system. The cell culture test is a highly sensitive system and seems promising as a tool for studying biological activity.

Influence of the Cutaneous Application of Ice on Isolation and Control of Single Motor Units in Humans

MARTHA ANNE CLENDENIN, M.S.
Department of Physiology

In this study an electromyographic technique has been used to evaluate the effect of a brief cutaneous application of ice on motor neuron excitability in human subjects. Approximately 80% of the forty-one subjects participating in the study were successfully trained to isolate a single motor unit in the right Bicepts Brachii muscle. Once the training period was completed, the subjects learned to control the single unit as evidence by the electromyographic record of his response to a start-stop command, to a random number sequence, and in synchrony with the sound of the metronome. The subject's success in learning to isolate and control a single motor unit to these experimental tasks was observed to be dependent on his concentrated volitional effort.

When the subject learned to perform these tasks accurately, ice in the form of a cube was applied by light stroking to the skin area overlying the Biceps muscle until an even erythema was evident. Immediately after the ice application the subjects repeated the learned experimental tasks, and the influence of afferent stimulation on isolated motor unit activation and control was evaluated. The cutaneous application of ice affected the subject's performance of these tasks as evidenced by facilitation of background motor unit activity and spontaneous firing of the originally isolated motor unit. This facilitation was observed in approximately 60% of the subjects during a start-stop sequence and during the activation of the isolated motor unit in synchrony with the sound of the metronome, and in 50% of the subjects during a random number sequence.

It is suggested that the method of ice application used in this study caused facilitation of motor unit activity due to the excitation of all types of cutaneous cold thermoreceptors, mechanoreceptors, and proprioceptive receptors. Further studies are necessary in order to identify the influence of each of these receptor types on the excitability states of motor neurons within the central nervous system.

Sodium Dodecyl Sulfate Inactivation of Bovine Liver Glutamate Dehydrogenase

STANLEY CLARK YUSKO, M.S.
Department of Biochemistry

The physical and catalytic effects of the detergent, sodium dodecyl sulfate, on the enzyme, bovine liver glutamate dehydrogenase, were examined by physical and kinetic methods. Double reciprocal plots of velocity versus substrate concentration indicated that low concentrations of sodium dodecyl sulfate gave uncompetitive inhibition. It was also determined from Hill plots of activity versus inhibitor concentrations that there was probably cooperativity in detergent binding. Further studies using the competitive inhibitor, isophthalate, showed that binding of substrate to the active site of glutamate dehydrogenase was not changed by the presence of detergent. Molecular sieve studies showed that there was no change in the size of the enzyme at low detergent concentrations that produced uncompetitive inhibition. However, a higher concentration of detergent, which caused complete inactivation, was found to dissociate and alter fluo-

rescence of the enzyme molecule. These data indicate that sodium dodecyl sulfate affects glutamate dehydrogenase by two different mechanisms. Sodium dodecyl sulfate, at low concentrations, is an uncompetitive inhibitor. At higher detergent concentrations, denaturation and protein dissociation occur.

A Study to Determine why a Group of Knowledgeable Women Did or Did Not Seek a Regular Cervical Smear Examination

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School of Nursing

The purpose of this study was to determine why a group of knowledgeable indigent and medically indigent women did or did not seek regular cervical smear examination. Special emphasis was placed on how knowledge regarding the cervical smear examination was obtained in an attempt to determine which sources and methods of health teaching were effective or ineffective in motivating women to seek the smear examination regularly.

An interview schedule was utilized with twenty-five subjects. All had recently received or were receiving treatment for cervical cancer. Fifteen subjects had neither sought nor been subjected to the cervical smear examination regularly. The remaining ten had either sought or been subjected to the cervical smear examination regularly.

The results of the study revealed the following: (1) Personal contact seemed to be the best motivator for seeking cervical smear examinations but was most effective when the contact was an individual whose opinion was highly valued; (2) In general, indigent women who learned about the smear examination from mass media, friends or relatives were not favorably influenced to seek examination; (3) Indigent women have been subjected to cervical smear examination without any explanation of the procedure by the health professionals in attendance; (4) Those subjects who sought examination regularly seemed to be motivated by a fear of cancer and the desire for peace of mind.

A Study to Determine the Effects of Deliberate Nursing Intervention upon Postoperative Pain in a Selected Group of Male Patients

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This exploratory study investigated the effects of deliberate nursing intervention upon postoperative pain in a selected group of male patients. Six adult males who underwent thoracotomy were selected as subjects for the study. Six additional patients, randomly selected from hospital records from the previous year, comprised the control group.

The investigator acted as each patient's nurse giving care during the 3–11 PM tour of duty on the operative day and for the first two postoperative days. When the patient complained of pain, the investigator initiated verbal exchange to determine the nature of the pain and for the patient to select the methods of relief to be employed. Patients chose either prescribed analgesics or deliberate nursing intervention.

At the time of the initial complaint, baseline recordings of the patient's blood pressure, pulse and respiratory rate were recorded. Thirty and sixty minutes after the selected intervention these parameters were again measured and verbal exchange took place between the patient and investigator. Intervention was considered effective when patient's verbal response indicated relief and or when the physiological parameters were decreased from the pre-intervention level.

In eleven out of twelve pain occurrences the blood pressure and pulse were both reduced from the pre-intervention level regardless of the method of intervention. Due to the nature of surgery the patients were subjected to, it is impossible to speculate concerning alterations in the respiratory rate as an indicator of pain relief.

In this small sample, medication was selected as a method of relief six times and nursing intervention was selected six times. The medication was ineffective in relieving pain four out of six times as evidenced by the patient's verbal responses. Deliberate nursing intervention was effective in relieving pain six times out of six as evidence by the physiological parameters and verbal responses of the patients. Deliberate nursing intervention was more effective than medication in relieving pain.

Analysis of data revealed that patients in the experimental group required less medication than those patients in the control group. The control group re-

ceived almost five times the medication as the experimental group.

Obvious limitations included a small highly selected population; the absence of a second investigator to eliminate observer bias and the interrupted time interval of study. Replication of this study is recommended in order to verify the contribution of deliberate nursing intervention to postoperative pain relief.

A Study to Ascertain the Relationship of Certain Factors as they Contribute to Lack of Control or Improper Management of Diabetic Patients Admitted to In-Patient Hospital Service

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It was the purpose of this study to ascertain the relationship of selected factors as they contributed to lack of control and/or inappropriate body weight of adult diabetic patients admitted to in-patient hospital service. These factors were knowledge of the diabetic regimen, practice of the regimen, and knowledge of the relationship between practice of the diabetic regimen and symptoms and/or complications of diabetes.

Two hypotheses were formulated. It was predicted that patients having incorrect or limited knowledge of the regimen would not practice their regimen correctly and would have limited knowledge of the relationship between practice and symptoms and/or complications of diabetes. It was also predicted that more patients having knowledge of the regimen and knowledge of the relationship between practicing the regimen and symptoms and/or complications of diabetes would practice more of the regimen correctly than those patients with knowledge of the regimen but without knowledge of the relationship.

Data were collected by three means. Descriptive data were obtained from the current hospital record. An interview schedule which incorporated observation was constructed to include questions to elicit the patient's knowledge, practice of the regimen, and knowledge of the relationship between practice and symptoms and/or complications of diabetes. Socio-economic data were also obtained.

During a ten week period, staff or private hospitalized patients who were on insulin, between the ages of 21 and 65, diagnosed as being diabetic for at least six months, and out of control by predetermined criteria were studied. A total of 20 patients fulfilling these criteria were interviewed and observed for the selected practices of the diabetic regimen.

Socio-economic and descriptive data for the twenty patients were tabulated. The scores for each patient in each category of the interview were calculated and the total scores derived. Correlation coefficients based on the patient's score in each category were determined to establish the possible existence of relationships between categories.

Due to the small study population only trends appeared in the data. The majority of the study population was Negro, female, and over 50. The median income and mean grade level of the study population was lower than the average for diabetics based on accepted national statistics. Total number of acceptable responses for the entire study population in all three categories of the interview schedule was low on a scale of 0–9. The mean total score indicated that the majority of the study population had less than 50 per cent acceptable responses and no patient had over 75 per cent or 20 acceptable responses out of a possible 27. The scores concerned with knowledge of relationship items were lowest with 14 patients having scores of 4 or less out of a possible 9.

Negro subjects had a higher number of unacceptable responses than did white subjects. Those who had been diabetic longer had the higher scores when the total scores were compared. Patients making the lower scores in each category of information had slightly lower levels of education, inability to read and write, and lower incomes than did the study population as a whole.

There was insufficient evidence to confirm the hypotheses. However, correlation coefficient results showed a significant relationship existed among all three categories of information requested on the interview schedule.

Enhanced Toxicity of Combinations of Bacterial Endotoxin with Antitumor Drugs

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Combinations of bacterial endotoxin with antitumor drugs resulted in an enhanced toxicity for mice. Sublethal doses of either pactamycin or sparsomycin given simultaneously to three strains of mice (BALB/sy, RFW, N.Y.S.R.) with sublethal doses of endotoxin killed a substantial proportion of the animals so treated.

The antihistamine chlorpheniramine and the non-steroidal anti-inflammatory agent, phenylbutazone, were unsuccessful in alleviating the pactamycin-endotoxin synergistic toxicity. Heparin, an anti-coagulant, was likewise found ineffective in reducing the toxicity associated with the sparsomycin-endotoxin synergy. Methyl-prednisolone, a potent anti-inflammatory steroid, was found to be successful in reducing the death associated with both sparsomycin-endotoxin and pactamycin-endotoxin synergies. It has been suggested that both sparsomycin and pactamycin be re-evaluated in combination therapy with anti-inflammatory steroids.

Pactamycin was found to be composed of more than one constituent when developed on silicic acid thin layer chromatographic strips in toluene-isopropanol-ethyl acetate (60:10:30). Presumptive evidence has been presented indicating a reduced clearance and/or detoxification of pactamycin when the drug is given in combination with endotoxin.

Death due to daunomycin was found to be a delayed process. When endotoxin was given to mice that had four days earlier been administered daunomycin, an increase in the death rate, indicative of a synergistic interaction, was observed.

Changing Personnel Requirements of a Modern Clinical Laboratory in the Face of Automation and Computerization

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Department of Pathology

The introduction of automated and semi-automated equipment into the clinical laboratory within the past few years has had a great impact upon its staffing requirements. The more recent introduction of computerized laboratory systems has created even greater demands for more highly specialized personnel. In order to determine what these demands are and how they affect the Medical College of Virginia, a study was made of the future plans of companies marketing this equipment and of the degree of automation presently established in the different sections of the laboratory. Another study was also made to determine how greatly automation has affected the laboratory workload from 1947 to the present time.

Since problems of personnel requirements are concentrated primarily around the registered medical technologist, the educational background and preparation of these persons was studied by surveying 872 catalogues from colleges offering baccalaureate degrees with a major in medical technology. The curriculums were compared as to courses required and recommended. Affiliation and non-affiliation of the colleges with schools of medical technology were noted. Performance of the students graduating from the School of Medical Technology, Medical College of Virginia, from 1953–1968 was surveyed as to the grades they achieved in college compared to those achieved in training on a year-to-year basis. Finally, a survey was made of current recruitment literature distributed by the Board of Registry of Medical Technologists of the American Society of Clinical Pathologists to determine whether or not the information contained therein was an accurate description of the personnel requirements of the clinical laboratory.

The following conclusions were drawn from the data presented in this study:

(1) Automated equipment for use in the clinical laboratory is becoming too complicated to be efficiently operated by persons now being trained in schools of medical technology. As a result, laboratories will soon need persons trained especially in the maintenance and operation of automated and computerized equipment.

(2) The impact of automation on the clinical laboratory at the Medical College of Virginia is not easily assessed since accurate records of the number of tests run by automated equipment and manual methods are not available. Also, the increase in workload cannot be studied for the same reason.

(3) An analysis of the data from the records of students graduating from the School of Medical Technology, Medical College of Virginia, from 1953–1968 reveals that there has been essentially no change in the ability of the students to achieve good grades, nor has there been any trend toward better or poorer grades from year to year.

(4) A survey of recruitment literature shows that most publications do not accurately describe the jobs performed by medical technologists in the modern clinical laboratory. As a result, persons who may be of great value in the laboratory, such as men who have an interest in electronic equipment, are not being attracted into the field of medical technology.

A proposed solution to the problem of providing well-trained personnel for the clinical laboratory is the extension of Whitehead's "hot" and "cold" laboratory concept which is currently being used at the Queen Elizabeth Hospital, Birmingham, Alabama. The "cold" laboratory consists of automated equipment and computers and is concerned mainly with routine laboratory testing. The "hot" laboratory employs more highly trained personnel who are concerned with manual testing procedures, "stat" procedures, and research. The establishment of "hot" and "cold" schools of medical technology would solve the manpower needs of both the small, 50-bed hospitals and the large,

1000-bed hospitals. "Hot" schools would be concerned with teaching good laboratory techniques and the theory behind each procedure taught. "Cold" schools would teach maintenance and servicing of automated equipment and computers and the theory behind their operation.

The future clinical laboratory will be a sophisticated set-up of both automated-computerized and manual methods of testing. Therefore, personnel of varied educational levels and training will be required to meet its demands.

Relationship of Antibodies to Cytomegalovirus Between Normal Blood Donors and Open-Heart Surgery Patients

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In a serological study of Cytomegalovirus (CMV) infection in the Richmond area, sera samples from 247 blood donors were tested for the presence of cytomegalovirus complement-fixing antibodies. Ages of the donors ranged from 19 to 61 years.

While 25% of the donors over 35 years of age had positive serum, the maximum incidence was found in the group composed of persons 54 to 61 years old (42%). Forty-three of the 247 samples of sera contained demonstrable complement-fixing antibodies (17.5%).

Thirteen patients who had open-heart surgery and received multiple fresh blood transfusions were studied. A four- and five-fold significant postoperative rise in the titer of complement-fixing antibodies to cytomegalovirus was found in two of the twelve surviving patients (16%). A two-fold rise in titer was found in a third patient, whose preoperative titer had been low. The cytomegalovirus was not isolated from blood or urine of the patients. None of the patients was reported to have any clinical manifestation of a cytomegaloviral disease, and no post-transfusion mononucleosis was recorded. The transmission of the infection via large amounts of transfused fresh blood and the possible reactivation of a latent or past infection are discussed.

A Nonequilibrium Thermodynamic Model of ION Transport in a Three-Compartment System

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Department of Physiology

A physical analog of steady-state sodium and potassium transport in a two-membrane, three-compartment system was studied utilizing the principles of nonequilibrium thermodynamics. This physical system is analogous to physiological systems where one compartment consisting of a cell monolayer separates two other compartments, such as the interstitial fluid and the renal tubule lumen in the kidney. The membranes in the model system serve only to localize the chemical potential gradients between compartments. The phenomenological equations relating the flows through the membranes to the chemical potential gradients were developed from the equation for energy dissipation within each membrane. The flows defined both the nonsteady-state rates of change of concentrations within each compartment and the steady-state transport across each membrane.

Ion transport due to chemical convection was studied by adding water to the "cell" compartment and removing it from the "interstitial" compartment. The "lumen" compartment was left as a strictly passive compartment. The Na^+, K^+, and Cl^- concentrations were measured periodically until a steady-state was reached.

In further experiments the concentrations of components in the "lumen" compartment were held constant by a constant flow of NaCl-KCl-H_2O solution through the compartment. The constant flow of water into the "cell" compartment distributed itself among both the "lumen" and "interstitial" compartments according to the mechanical filtration properties of each membrane. In initial experiments, the flows were unidirectional into the "interstitial" compartment. In later experiments the flow was distributed to both the "interstitial" and "lumen" compartment. After the system had reached a steady-state, the concentration of components, the flows in and out of the "lumen" compartment and the flow of water into the "cell" compartment were measured. The magnitudes and directions of the steady-state transport of components were determined.

The nonsteady-state experiments demonstrated a transient transport of Na^+, K^+, and Cl^- ions from the "lumen" compartment to the "interstitial" compartment against a concentration gradient. At low solvent fluxes the ion transport occurs with the concentration gradient. At intermediate solvent fluxes, K^+ and Na^+

are transported in opposite directions; K^+ is transported down a concentration gradient while Na^+ is transported against an equal or larger gradient.

Steady-state transport of Na^+ and K^+ from the "lumen" compartment to the "interstitial" compartment may be maintained by a solvent flux in the direction of transport. The magnitude of this transport is greatest when the concentrations of components in the two compartments are equal, and decreases as the concentration ratio of components in the "intestitial" compartment to those in the "lumen" increases. For the combinations of solvent fluxes and component concentrations investigated, the transport of K^+ was usually greater than the transport of Na^+.

The Effects of Alcohol on Three Levels of Complex Human Behavior

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Department of Physiology

The effects of alcohol on three levels of complex human behavior were studied in twelve male subjects between the ages of 21 and 35 using the LRC Complex Coordinator. Each level of complexity contained an increasing component indicative of cognitive behavior. The motor component of all three levels was maintained approximately constant. The blood alcohol concentrations studied were 0.000, 0.010, 0.050, and 0.100 percent, as determined with the Breathalyzer®. Alcohol was administered in the form of 50 percent ethanol mixed with frozen orange juice concentrate. All blood alcohol concentrations were studied in the same subject during one test session. The study was replicated. The study was repeated twice without alcohol.

Analysis of variance was performed on the data for ten subjects using as dependent variables the time to perform 100 problems and the total errors for all four limbs for 100 problems. The variability between subjects was significant for both the alcohol test sessions and the control sessions. The variability due to blood alcohol concentrations was significant only for the test sessions during which the subjects received alcohol. The variability due to complexity of the task was significant for both the alcohol and control test sessions. There was a component of variability which indicated that the subjects responded differently to the increasing complexity. When the time to perform 100 problems was analyzed, there was an indication that as the task became more complex the alcohol effect became more pronounced but this did not hold true when the total errors per 100 problems was analyzed. When the time to perform 100 problems was analyzed, there was a component of variability that indicated that all subjects responded in the same direction to increasing blood alcohol concentrations but this relation did not hold when the total errors was analyzed.

Data are presented which indicate that cognitive processes were not affected by these blood alcohol concentrations. Performance on all three tasks was affected significantly; however, the effect of the alcohol appeared to be on the subject's ability to make precision positioning movements of the limbs.

Exposure of Man to a Simulated Lunar Magnetic Environment, Physiological and Central Nervous System Effects

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Department of Physiology

The lunar magnetic field intensity at the surface was measured by Apollo 12 to be between 30 and 40 gamma. The geomagnetic field is approximately 0.5 gauss or about one thousand times more intense than the lunar field. The basic element of this study is the search for some aspect of human physiology which is coupled to the normal geomagnetic field and which would be significantly changed in the low intensity lunar magnetic field.

In previous experiments, six human subjects were exposed for ten days to a simulated lunar magnetic field generated by a coil system and two subjects were exposed for five days to a simulated lunar magnetic field created by a high permeability shield. Extensive physiological and psychological tests were conducted on these subjects. Only one test, scotopic critical flicker frequency (SCFF), indicated a possible physiological effect of the low field environment. The present study is a continuation of this previous work.

The coil system and shielded facilities were used to study a total of 18 subjects. The results indicate that psychological stress may be induced in some subjects by the necessary confinement associated with the experimental procedure; however, there is no indication that any physiological stress is induced by the low magnetic field environment. In conclusion, humans may be safely exposed to the lunar magnetic environment for up to 14 days; however, in view of previous studies by other authors in which a generalized hyperplasia was found in chronically exposed mice, the safety of humans is still in question for exposure periods exceeding 14 days.

SCFF apparatus was developed with the following

significant features: 1) a discontinuous exposure to the flickering stimulus without changing the size or intensity of the test area, thus permitting the subject to compare a flicker and fused condition continually throughout the measurement period, 2) the end point is reached when the subject sees no change in the test area even though the presentation is still discontinuous, 3) a constant rate of frequency advance, and 4) an active response by the subject for each flickering stimulus period.

A reaction time apparatus was developed which can provide a histogram in approximately ten minutes. The first sustained rise of the histogram was much more stable than the mean. A theoretical analysis of these histogram parameters indicates that the position of the first sustained rise is a function of the homeostatic state of the neuromuscular system and that the position of the mean relative to the first sustained rise is a function of the psychophysiological state of the central nervous system. This analysis is useful for chronic studies of these systems. It is also useful for studying the direct physiological influence and the indirect psychophysiological influence of a particular sensory input on the central nervous system.

Evaluation of the Essentiality of Dextran in the Dental Caries Process

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Department of Biochemistry

Dextran is an extracellular polysaccharide, an *alpha* 1,6′ polyglucose, synthesized from sucrose by some bacteria. Dextran has been postulated as a fundamental component in the dental caries process. The proposed role of dextran is that of an adhesive, extracellular agent which gives rise to bacterial tooth colonization. This hypothesis that dextran is an essential factor in the dental caries process has been evaluated experimentally.

A diet containing 10% high molecular weight dextran was fed to microbe-bearing rats to test the dextran hypothesis.

Lactobacillus casei 4646, an organism which induced dental caries in sucrose-fed, gnotobiotic rats but caused no dental plaque, was examined for an extracellular dextransucrase enzyme. Cell-free, extracellular preparations from *L. casei* 4646 culture fluids were assayed for dextransucrase by two methods:

1. A modified-Somogyi assay was employed to estimate the release of reducing sugar from sucrose by the extracellular, cell-free preparations.

2. A coupled-enzyme, spectrophotometric assay was developed to estimate fructose release from sucrose and to differentiate glucose from fructose release by the cell-free, extracellular preparations.

Sucrose-grown *Lactobacillus casei* 4646 cells were examined for extracellular dextran. *L. casei* 4646 cells, growing in a sucrose-containing, *in vitro* system, were tested for the ability to form visible, adherent layers of cells on a stainless-steel wire.

Dextran-fed rats did not experience tooth decay. Although cariogenic, *Lactobacillus casei* 4646 possesses no extracellular dextransucrase, does not synthesize extracellular dextran, and does not form visible, adherent layers of cells on the stainless-steel wire. From these and other considerations, it was concluded that dextran was not essential in the dental caries process.

Methylurea—Its Intermediary Role in the Physiological Disposition of Methylamine

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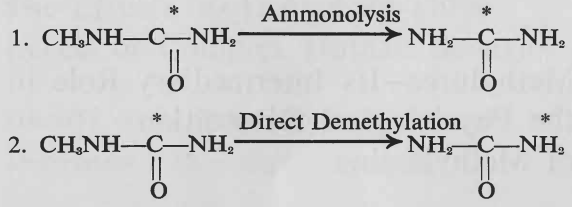
Evidence has been presented to show that the metabolism of the compound leads to the formation and excretion of methylurea, a metabolite suspected but never conclusively shown to exist by previous investigators.

Urinary methylurea, after the administration of methyl-^{14} C-amine, was shown to have ^{14}C-activity at both the carbonyl and methyl portions (groups) of the molecule. The presence of the ^{14}C-activity in the carbonyl group of methylurea was derived from the same metabolic pool of carbon dioxide that is used in the formation of urinary urea and respiratory carbon dioxide. Administration of methyl-^{14} C-amine led to the formation of respiratory CO_2-^{14}C, as noted by various investigators, and it was shown that during a 24-hour period the ratio of specific activity, urea/respiratory CO_2, approached one, in agreement with previous data reported by Mackenzie and du Vigneaud presented for the rat after administration of L-methionine-methyl-^{14}C.

In order to quantitatively investigate some aspects of metabolism of methylurea a procedure was developed for the convenient synthesis of N-methyl-^{14}C-urea and N-methylurea-^{14}C-carbonyl. Here advantage was taken of the Wurtz reaction, utilizing methyl-^{14}C-amine and potassium cyanate-^{14}C respectively. The reaction mixture was then passed through cationic and anionic exchange resins to obtain a virtually pure product.

In a series of experiments in which N-methylurea-^{14}C-carbonyl and N-methyl-^{14}C-urea were injected intraperitoneally, the urinary urea of the treated animals was examined for radioactivity. Limited amounts of radioactivity were found in the carbonyl group of urea following administration of N-methyl-^{14}C-urea. The appearance here of ^{14}C-activity is attributed to the enrichment of the carbon dioxide pool with ^{14}C through oxidation of the ^{14}C-methyl group. After administration of N-methylurea-^{14}C-carbonyl, urinary urea contained a 200-fold excess of ^{14}C-activity, based upon the measurement of the specific activity of the respiratory carbon dioxide. These data indicate a metabolism of methylurea to urea which is accomplished in such a manner as to maintain the integrity of one or more of the nitrogen carbon bonds of methylurea.



* isotopic

The data presently available do not permit exclusion of either route as a participating factor in the demethylation of methylurea, and point to the necessity for additional studies for complete elucidation of the mechanisms involved.

In addition to providing new routes for the mammalian metabolism of methylamine through a proposed methylurea cycle, this dissertation provides indirect evidence to demonstrate that the effect of iproniazid as an inhibitor of methylamine oxidation is mediated through enzyme systems separate from monoamine oxidase systems which have been invoked as major contributors to metabolism of methylamine by other investigators.

The Adrenergic and Cholinergic Innervation of the Autotransplanted Canine Kidney

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Until recent years, it was assumed that severing the renal blood vessels and ureter during transplantation would, in effect, denervate the kidney. According to some investigators, transplantation may not produce total denervation, for some intact renal reflex arcs may remain; several small hilar and intrarenal ganglia might be distal to the line of vascular division and suture.

No studies using modern histochemical methods have been performed on transplanted kidneys; rather, the technique of stripping the renal vessels and ureter has been used. This "denervating" method has been the subject of criticism due to the possibilities of an incomplete stripping procedure.

In addition to the lack of information regarding the innervation of renal transplants, there exists an abundance of controversial descriptions of normal renal innervation. Knowledge regarding the intrinsic innervation of the kidney was based for years on experiments using various standard nerve staining techniques, particularly silver impregnation and methylene blue. Recently, there have been reports on the application of newer histochemical methods to the study of renal innervation.

Due to the existence of controversial descriptions of renal innervation and the paucity of information regarding the innervation of transplanted organs, this investigation was undertaken. This study directly concerned the normal intrinsic distribution of the renal nerves and what effects are imposed on these nerves by autotransplantation. The following points were investigated: (1) the normal distribution of intrarenal neuronal elements using modern histochemical techniques, (2) if total degeneration of adrenergic and cholinergic nerves does indeed occur following transplantation, (3) if and when regeneration of adrenergic and cholinergic fibers occurs, (4) if the acetylcholinesterase (AChE) activity in the glomerulus is due to nervous or non-nervous elements, and (5) if discrete hilar ganglia exist.

Nineteen mongrel dogs were used in transplantation studies in which one kidney was autotransplanted to the ipsilateral iliac fossa. Every animal was anesthetized with 30mg/kg sodium pentobarbital administered intravenously. The dogs were all females which varied in weight from 15 to 18 kilograms. The severed renal vessels were anastomosed to the common iliac artery and vein. One operation was performed in which identical surgical procedures were followed with the exception of actually transplanting the kidney. This sham-operated kidney, as well as the left kidney in each dog, served as controls.

To investigate the existence of autonomic ganglia in the hilum, 7 normal kidneys were removed from normal dogs. The hilar regions of each kidney was removed and was examined using the following histological techniques: hematoxylin and eosin, luxol fast blue—P.A.S., Holmes' silver impregnation, and the Koelle thiocholine method. The catecholamine levels in two 2-week post-transplant kidneys were also determined. Catecholamine assays were run in duplicate on renal cortex samples of approximately 5g. Normal kidneys served as controls.

The evaluation of experimental results regarding transplanted kidneys was accomplished by using two specific histological techniques. To determine the presence of adrenergic nerve fibers, the fluorescence technique of Falck was employed. Cholinergic fibers were identified using a modification of the thiocholine technique of Koelle.

Both adrenergic and cholinergic nerve fibers were observed only in relation to the preglomerular arterial vessels. The nerves were seen to form a dense plexus immediately outside the tunica media of the vessels. In addition, larger nerve bundles were present in the connective tissue sheaths of the renal, interlobar, and arcuate arteries. Adrenergic and cholinergic nerves were also seen coursing with the vasa recta. Only adrenergic fibers could be observed in relation to the large veins.

Following transplantation there was a total degeneration of adrenergic fibers within one week. Catecholamine levels fell to negligible amounts post-transplant. In only one case was regeneration of a few adrenergic fibers noted. Conversely, total degeneration of cholinergic fibers was observed in only 3 of 11 transplants; the other 8 showed variable degrees of degeneration, from almost total to very little. AChE activity was apparent in glomeruli but was greatly diminished or abolished by renal perfusion with normal saline before normal histologic procedures were begun. Positively-stained erythrocytes were regarded as being responsible for the staining seen. Discrete autonomic ganglia which were intensely AChE-positive were seen in the renal hila. It is believed these AChE-positive ganglion cells (also found scattered along hilar nerve bundles) are the source of the nerve fibers which remain intact and viable following vascular division during transplantation. Therefore, it is suggested that "total denervation" should refer to intrinsic as well as extrinsic innervation.

Studies on the Carboxyl-Terminal Tetrapeptide Sequence of Bovine Serum Albumin

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Although bovine serum albumin (BSA) has been used widely in scientific investigations, little is known about its amino-acid sequence. Only the dipeptide sequence in the carboxyl-terminal region has been determined, while the amino acid composition through the hexapeptide has been reported. This composition and sequence were determined by digestion of BSA with the hydrolytic enzyme, carboxypeptidase, and were reported as (Ser, Val)-(Thr, Ala)-Leu-Ala. In addition, peptide fragments from BSA have been hydrolyzed with carboxypeptidase and the sequence of one of these fragments has been related to that of the in-tact protein.

Based upon the evidence cited above, it was surmised that small peptides with the same amino-acid composition as that in the carboxyl-terminal region of BSA might be employed advantageously to further enhance our knowledge of this C-terminal sequence of BSA. These peptides could be hydrolyzed with carboxypeptidase and the rates of release of the amino acids could be determined and compared with the release rates obtained for the amino acids from the carboxyl-terminal region of BSA when both were digested under identical conditions.

Three hexapeptides, each with a sequence that might match that of the carboxyl-terminal hexapeptide sequence of BSA, were prepared by the solid-phase method of peptide synthesis, which was developed by R. B. Merrifield at the Rockerfeller University. The three synthetic peptides contained an alanyl residue at the carboxyl-terminus because this residue was assigned that position in BSA by several investigators. These peptides were prepared in yields of 65–72% and were then isolated and purified by gel-filtration chromatography. Yields of the purified peptides were 46–49%.

These synthetic hexapeptides were hydrolyzed with carboxypeptidase as was the protein, BSA. The free amino acids in each of the hydrolyzates were determined periodically over a 29-hour period. These data were then plotted as the ratios of alanine to leucine and of alanine to threonine as a function of time, for each of the peptides, and for albumin. Comparison of the curves and other available data leads to the conclusion that the carboxyl-terminal tetrapeptide sequence of BSA is Thr-Ala-Leu-Ala.

In addition to the hexapeptides, three pentapeptides and three tetrapeptides were synthesized. All of the synthetic peptides were studied instrumentally, with infrared, nuclear magnetic resonance, optical rotatory dispersion, and circular dichroic spectroscopy, to determine what conformation these peptides assume in the solid state and in solution. It was concluded from the infrared spectral studies that all of the peptides in the solid state assume an extended form which is characterized by strong intermolecular hydrogen bonding. Optical rotatory dispersion data lead to the conclusion that in water solution the peptides also assume an extended conformation. No evidence was obtained to suggest an onset of helix formation. The optical rotatory dispersion spectra of the peptides were characterized by negative plain curves. The dispersion constants for most of the peptides investigated were in the

range of 216 ± 2 nm. The circular dichroic spectra of the peptides were characterized generally by negative minima in the range of 220–229 nm.

Nuclear magnetic resonance spectroscopic data could not be interpreted reliably in terms of peptide conformations, but they did contain information relative to exchangeable protons in the peptide structures. In addition, these data provided information concerning hydrogen-hydrogen interactions within the various peptide structures.

Substituent Effects on Cholinergic Activity of Substituted Benzyltrimethylammonium Salts

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Department of Chemistry and Pharmaceutical Chemistry

In recent years, physico-chemical methods of determining structure-activity relationships of biological agents have supplemented classical chemical and pharmacological methods. Physico-chemical methodology has previously been applied to problems concerning the muscarinic and nicotinic receptors of the cholinergic portion of the autonomic nervous system. However, the nature of the structure of many muscarinic and nicotinic agents has limited the application of physico-chemical methods. Thus the study of the nature of cholinergic receptors has been hindered by a technical incompatibility in methodology. With this problem in mind, two series of cholinergic agents have been designed to permit the simple determination of some of their physico-chemical properties. These properties would possibly be correlatable to the pharmacological activity of the compounds. Two series of substituted benzyltrimethylammonium salts have been designed to fulfill the "five-atom rule" of muscarinic activity.

Of the compounds prepared and tested as muscarinic agonists on rat jejunum, only two demonstrated muscarinic activity. Seven of the compounds tested possessed curariform activity as measured by rat diaphragm paralysis. The low order of activity of these compounds precluded any correlation of biological activity with physico-chemical parameters. It was noted that the "five-atom rule" does not appear to be valid for this particular series of compounds.

The Role of the Reticuloendothelial System in Host Defense

ALBERT ENOCH MUNSON, PH.D.

Department of Pharmacology

The studies reported here examine the organism's defenses to better understand the RES and its participation in protection of the host to the external environment. The RES was studied by using agents which modify the phagocytic activities of the fixed macrophages of the liver, lung and spleen and by evaluating the intravascular clearance, organ uptake, immunologic response and host defense to microorganisms, tumors and drugs during various functional phagocytic states of the RES.

Inbred male albino mice (NYLAR) were used throughout the studies. *S. aureus, D. pneumoniae, C. neoformans* and *T. duttoni* were the microorganisms used and Erhlich adenocarcinoma was the tumor studied. The metabolism of hexobarbital was used to measure the microsomal oxidase status during RES modification.

Vascular clearance and organ uptake of ^{51}Cr sRBC and ^{131}I "RE Test Lipid Emulsion" showed similar clearance patterns as colloidal carbon but showed different organ distribution patterns. Pyran copolymer, a synthetic polyanion and a known antitumor and interferon inducing agent was the RES modifying agent studied. The functional state of the RES during the biphasic response (blockade of particulate uptake followed by stimulation of uptake) produced by pyran copolymer pertained only to vascular clearance and hepatic uptake of the injected particulates. The localization of sRBC and the "RE Test Lipid Emulsion" into the spleen and lungs was independent of the biphasic response. This indicates that vascular clearance and organ uptake of more than one size of particulate is necessary to more fully evaluate the functional status of the RES.

The 19S immunoglobulin response to sRBC, as measured by hemolysin titer and Jerne plaque technique, correlated most closely with the amount of sRBC phagocytized by the spleen and was not related to the phagocytic status of the liver. Stimulation of hepatic and splenic phagoctyosis was associated with increased host defense against *D. pneumoniae, S. aureus* and *T. duttoni.* The pulmonary macrophages may also have played an important role in the resistance to *C. neoformans.* The development of the Erhlich adenocarcinoma was markedly decreased in mice undergoing RES stimulation and this tumor inhibition may be the result of an enhanced immune response.

In regard to drug metabolism, modification of the

RES whether depressed or stimulated resulted in a marked inhibition in the metabolism of hexobarbital. This inhibition appears to be noncompetitive in nature. Stimulation and depression of the metabolism of hexobarbital by SKF 525A and chlorcyclizine produced no changes in the functional status of the RES. Chlorcyclizine given in a protocol to enhance drug metabolism reverses the depressant effects of zymosan on hexobarbital metabolism. SKF 525A given in a protocol to inhibit drug metabolism summates with pyran copolymer on the inhibition of hexobarbital metabolism. This additive effect may be the summation of the competitive action of SKF 525A and the noncompetitive activity by pyran copolymer. The noncompetitive activity is thought to be mediated through or by the Kupffer cell.

The RES is a multicompartmented system made up of various cells in those organs whose function includes monitoring and removing from the internal environment substances from within, such as old or damaged erythrocytes, tumor cells, etc. or substances derived from the exterior, such as bacteria, fungi and viruses. Although all macrophages have the ability to phagocytize, it appears that their is a division of labor in regard to other functions ascribed to the RES. For example, the liver represents a highly efficient filtering system of macrophages which can remove foreign materials from the circulation and in most cases catabolizes and excretes the phagocytized material. While alveolar macrophages patrol the pulmonary tissue for inhaled particulates and carry these to the lymphatic system for removal or antibody processing.

The splenic and lymph node macrophages are primarily concerned with antibody processing. After phagocytizing the antigen, the macrophage is believed to form an RNA or RNA-antigen complex which is passed on to the lymphocytic type cells which make antibody. Antibody, acting as an opsonin, unites all phagocytic cells in their ability to remove, destroy and excrete foreign antigens.

The Application of Absorbents and of Complexing Agents in Urolithiasis

CHARLES EDWARD O'REAR, PH.D.

Department of Chemistry and Pharmaceutical Chemistry

In a review of the literature on the causes of calcium oxalate urolithiasis the concept of a critical cluster has been used as a unifying principle in stone formation. Matrix materials could promote the formation of such critical clusters and (or) prevent their disintegration.

There is no established satisfactory treatment for calcium ovalate stone formers. The control of urinary pH is neither practical nor effective. The use of low oxalate diets, low and high phosphate diets, the administration of magnesium salts, and the irrigation of catheterized patients with citrate buffers and salts of EDTA have proven to be of marginal help in preventing or slowing down the growth of oxalate stones.

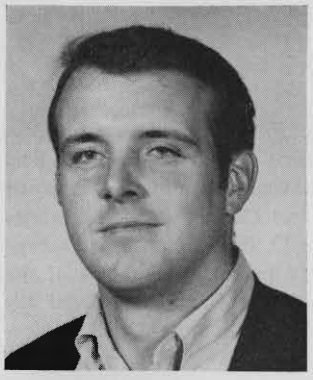
The foreign body technique for producing experimental urolithiasis in rats is suitable for magnesium ammonium phosphate. Less consistent results were obtained in calcium oxalate lithiasis produced by the administration of small doses of ethylene glycol. Attempts to produce larger amounts of calcium oxalate deposits on foreign bodies failed. It was found that deposition of oxalate crystals may occur both on a foreign body in the bladder and in the tissue of the kidney. Therefore, a more suitable model of calcium oxalate lithiasis was developed with the use of histopathological evaluation of kidney slices for tissue damage and the amount of calcium oxalate crystals.

Methylene blue or Vitamin C had little, if any, effect on the growth rate of deposit of calcium oxalate. A combination of these two compounds showed a synergic effect. Results with Azure A and New methylene blue and the *in vitro* adsorption studies of the alphazurin series suggests favorable results in inhibition of deposits by increased size of substituents on the amino nitrogen of methylene blue.

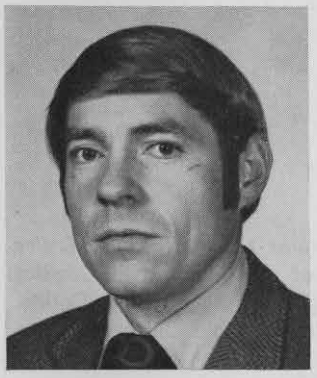
The tetramethyl ester of EGTA showed promise in the treatment of oxalate urolithiasis. Experiments using larger doses of the ester in which the EGTA/Ca ratio in urine can become greater than one may have a significant inhibiting effect in oxalate urolithiasis.

The administration of magnesium salts can be quite disastrous to the test animal. Magnesium can protect the animal in preventing calcium oxalate lithiasis but unfortunately large amounts of magnesium ammonium phosphate are produced.

Contributors



Walter H. Carter, Jr. *(A Note on the Probability of Misclassifying Students on the Basis of a Multiple Choice Examination; The Use of Principal Component Analysis to Increase the Ability of Multiple Choice Examinations to Distinguish Among Students)* is assistant professor of biometry at the Medical College of Virginia. After graduating with a B.S. degree in mathematics and physics from the University of Richmond, Dr. Carter attended the Virginia Polytechnic Institute and State University where he received his M.S. in mathematics and Ph.D. in statistics. His research interests include multiple response ridge analysis, the application of statistics to legal problems, and estimation from grouped populations.

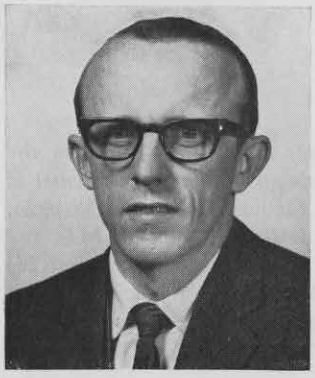


Roger E. Flora *(The Use of Principal Component Analysis to Increase the Ability of Multiple Choice Examinations to Distinguish Among Students)* is assistant professor in the department of biometry, Medical College of Virginia. He received his B.A. degree from the University of Virginia, and his M.S. and Ph.D. from the Virginia Polytechnic Institute and State University. Prior to his appointment at MCV, he was assistant professor of preventive medicine at West Virginia University. Dr. Flora's research interests are in the area of multivariate statistical analysis.

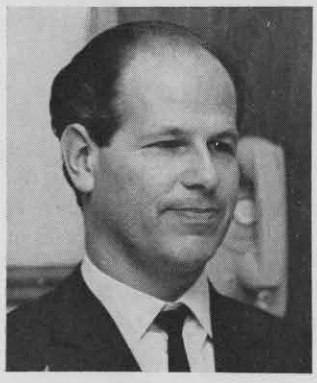
Kenneth E. Guyer, Jr. *(A Survey of Laboratory Programs for First Year Medical Students)* did his undergraduate study in chemistry at the University of Texas at Austin. After receiving his M.S. and Ph.D. degrees from Ohio State University, he was a fellow in physiological chemistry at the Johns Hopkins School of Medicine. In 1964 Dr. Guyer came to the Medical College of Virginia, where he is assistant professor of biochemistry.

S. J. Kilpatrick, Jr. *(Alternative Methods of Grading One or More Multiple Choice Examinations; Collusion in Multiple Choice Examinations)* is chairman of the department of biometry at the Medical College of Virginia. He graduated from the Queen's University of Belfast, Northern Ireland, and did post-doctoral training at Iowa State University. Before joining the faculty at MCV, Dr. Kilpatrick was a lecturer in the departments of statistics and social medicine at Aberdeen University, Scotland.

Peter Mamunes *(New Developments in Screening for Inborn Errors of Metabolism)* is associate professor in the department of pediatrics, Medical College of Virginia. Before coming to MCV he was assistant professor of pediatrics at the New Jersey College of Medicine and Dentistry. Dr. Mamunes received his B.A. from Cornell University and his M.D. from Seton Hall College of Medicine. He did his internship and first year of pediatric residency at the University of California San Francisco Medical Center, and completed his residency at Babies Hospital, Columbia–Presbyterian Medical Center, New York City.



James L. Poland *(A Survey of Laboratory Programs for First Year Medical Students)* is assistant professor in the department of physiology at the Medical College of Virginia. After receiving his B.S. degree at Waynesburg College, Waynesburg, Pennsylvania, Dr. Poland went to West Virginia University where he received his M.S. and Ph.D. degrees.



Peter Reizenstein *(Pathogenesis of Secondary Anemia)* completed his medical studies at the Karolinska Institute in Stockholm, Sweden, and then worked at the Brookhaven National Laboratory. As head of the hematology section at the Karolinska Institute since 1967, his main interest has been the metabolism of erythropoietic factors in nutritional anemias.

Hugo R. Seibel *(A Survey of Laboratory Programs for First Year Medical Students),* a native of Radautz, Romania, is assistant professor of anatomy at the Medical College of Virginia. After his graduation from Brooklyn College, he continued study toward his M.A. there and at the University of Cincinnati. Dr. Seibel received his Ph.D. from the University of Rochester, where he was also instructor of gross anatomy until coming to MCV in 1967.

Diagnosis: spasm reactor

Decision: Donnatal®

	each tablet, capsule or 5 cc. teaspoonful of elixir (23% alcohol)	each Donnatal No. 2	each Extentab
hyoscyamine sulfate	0.1037 mg.	0.1037 mg.	0.3111 mg.
atropine sulfate	0.0194 mg.	0.0194 mg.	0.0582 mg.
hyoscine hydrobromide	0.0065 mg.	0.0065 mg.	0.0195 mg.
phenobarbital (warning: may be habit forming)	(¼ gr.) 16.2 mg.	(½ gr.) 32.4 mg.	(¾ gr.) 48.6 mg.

Brief summary. Side effects: Blurring of vision, dry mouth, difficult urination, and flushing or dryness of the skin may occur on higher dosage levels, rarely on usual dosage. Administer with caution to patients with incipient glaucoma or urinary bladder neck obstruction as in prostatic hypertrophy. Contraindicated in patients with acute glaucoma, advanced renal or hepatic disease or a hypersensitivity to any of the ingredients.

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Contraindications: Patients with known hypersensitivity to the drug.

Warnings: Caution patients about possible combined effects with alcohol and other CNS depressants. As with all CNS-acting drugs, caution patients against hazardous occupations requiring complete mental alertness (*e.g.*, operating machinery, driving). Though physical and psychological dependence have rarely been reported on recommended doses, use caution in administering to addiction-prone individuals or those who might increase dosage; withdrawal symptoms (including convulsions), following discontinuation of the drug and similar to those seen with barbiturates, have been reported. Use of any drug in pregnancy, lactation, or in women of childbearing age requires that its potential benefits be weighed against its possible hazards.

Precautions: In the elderly and debilitated, and in children over six, limit to smallest effective dosage (initially 10 mg or less per day) to preclude ataxia or oversedation, increasing gradually as needed and tolerated. Not recommended in children under six. Though generally not recommended, if combination therapy with other psychotropics seems indicated, carefully consider individual pharmacologic effects, particularly in use of potentiating drugs such as MAO inhibitors and phenothiazines. Observe usual precautions in presence of impaired renal or hepatic function. Paradoxical reactions (*e.g.*, excitement, stimulation and acute rage) have been reported in psychiatric patients and hyperactive aggressive children. Employ usual precautions in treatment of anxiety states with evidence of impending depression; suicidal tendencies may be present and protective measures necessary. Variable effects on blood coagulation have been reported very rarely in patients receiving the drug and oral anticoagulants; causal relationship has not been established clinically.

Adverse Reactions: Drowsiness, ataxia and confusion may occur, especially in the elderly and debilitated. These are reversible in most instances by proper dosage adjustment, but are also occasionally observed at the lower dosage ranges. In a few instances syncope has been reported. Also encountered are isolated instances of skin eruptions, edema, minor menstrual irregularities, nausea and constipation, extrapyramidal symptoms, increased and decreased libido—all infrequent and generally controlled with dosage reduction; changes in EEG patterns (low-voltage fast activity) may appear during and after treatment; blood dyscrasias (including agranulocytosis), jaundice and hepatic dysfunction have been reported occasionally, making periodic blood counts and liver function tests advisable during protracted therapy.

Usual Daily Dosage: Individualize for maximum beneficial effects. *Oral*—Adults: Mild and moderate anxiety and tension, 5 or 10 mg t.i.d. or q.i.d.; severe states, 20 or 25 mg t.i.d. or q.i.d. Geriatric patients: 5 mg b.i.d. to q.i.d. (See Precautions.)

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