Combined Tetanus-Diphtheria Immunization of Adults: Use of Small Doses of Diphtheria Toxoid

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Urgent questions of adult immunity to diphtheria may present themselves upon occasion in any health jurisdiction—among such groups as institutional personnel, staff workers with children, camp counselors, and the like. There is solid evidence here to guide the administrator in meeting such problems.

Immunization of children against diphtheria has been established as an essentially routine procedure. The majority of children in the United States are Schick-positive; reactions to diphtheria toxoid in children are few; and the use of standard doses of toxoid without prior Schick testing is therefore an effective and satisfactory method of diphtheria immunization in this age group.

The American adult, however, presents a different problem. No longer as generally immune to diphtheria as his parents were, he is nevertheless still prone occasionally to show untoward reactions to diphtheria toxoid (e.g.[1]). Incidence of such reactions can be reduced by either (1) immunizing only the susceptibles as determined by Schick testing, (2) use of purified toxoid, or (3) reducing the dose of toxoid administered. The first method is tedious and time-consuming and involves a tremendous amount of organization and labor when large groups are involved. In fact, it is widely felt that Schick testing is even more inconvenient than toxoid injections in adults, although it does reduce reactions. The second method is of great value, but it is by no means sufficient to eliminate all severe reactions.[2] The third method has attracted several investigators, but it is open to the logical question: Will small doses of toxoid provide a sufficient antigenic stimulus to protect the recipient?

Danish and Canadian investigators were the first to explore this approach systematically. Scheibel and Tulinius[3] showed that as little as 1 Lf of diphtheria toxoid, adsorbed onto aluminum

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This paper was presented in part before the Epidemiology Section of the American Public Health Association at the Eightieth Annual Meeting in Cleveland, Ohio, October 24, 1952.

This investigation was done in part under the sponsorship of the Commission on Immunization, Armed Forces Epidemiological Board, and was supported in part by the Office of the Surgeon General, Department of the Army. Most of the laboratory work was carried out at Boston University School of Medicine while the senior author was on the staff at that institution.

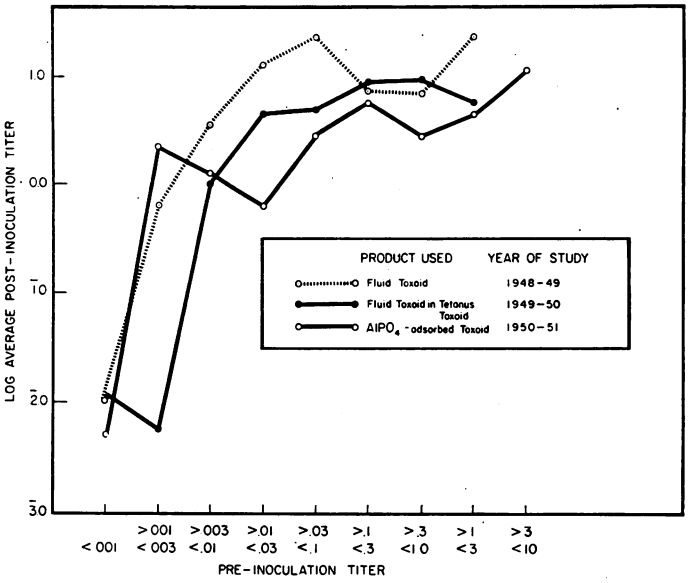

Figure 1—Preinoculation vs. postinoculation titers following injection of 1 Lf diphtheria toxoid

hydroxide, and given in two primary doses with a third dose one year later (i.e., a total dose of 3 Lf) produced an adequate antibody level in all of a small group of 15 previously susceptible subjects. Sellers, et al.,[4] employed a combined typhoid-paratyphoid-tetanus antigen containing 2 Lf diphtheria toxoid per ml. in fluid form, changing later to 4 Lf in adsorbed form.[5] With the latter preparation, 84 of 86 subjects had detectable antitoxin titers after three doses given a month apart. More recent work of both groups has considerably extended the above results.[6]

Our studies began in 1948 with an evaluation of the relationship between the dose of diphtheria toxoid and the response obtained when a single inoculation was employed.[7] The results indicated that one could generally obtain an immune response with as little as 1 Lf, but that less than this would markedly reduce the proportion of subjects in whom satisfactory antitoxin levels were induced. Meanwhile, a study of the reactions induced by diphtheria toxoid[2] had shown that even as little as 5 Lf of purified toxoid could induce severe general reactions in a small number of subjects. The 1 Lf dose appeared therefore to be about optimal for field trials on immunization of young American adults. The general efficacy of this dose in inducing an immune response in a large proportion of such subjects was meanwhile demonstrated by a series of studies in medical students * using fluid toxoid alone, fluid diphtheria toxoid in a tetanus toxoid menstruum, or diphtheria toxoid adsorbed on aluminum phosphate (Figure 1). Contrary to the general impression, we found that the adsorbed toxoid appeared to induce no more reaction than the fluid preparation, and, if anything, somewhat less (Table 1), although the antibody responses were comparable (Figure 1).

* We would again like to express our appreciation to the directors of health and especially to the students of Harvard Medical School, Tufts College Medical School, and Boston University School of Medicine for making these studies possible.

Table 1—Reactions to 1 Lf Diphtheria Toxoid

Degree of Reaction	Number of Subjects			
	Local		General	
	FTT *	APT †	FTT	APT
None	5	39	42	71
Mild	27	31	8	2
Moderate	14	4	5	0
Severe	10	1	1	2
Total	56	75		

* FTT = fluid toxoid, in tetanus toxoid
† APT = $AlPO_4$ — adsorbed toxoid

The safety and efficacy of small doses of aluminum-phosphate adsorbed diphtheria toxoid were then determined by their administration to volunteer service school personnel at Great Lakes Naval Training Center. Two series of such studies carried out on young adults found Schick-positive on two tests three weeks apart [8] had shown that approximately four-fifths of these apparently susceptible subjects developed protective antitoxin levels three weeks after two doses of 1 Lf of adsorbed diphtheria toxoid three weeks apart. A third dose, five months after the two primary doses, converted five out of a small group of seven who were still available for study at this time. The complete absence of reactions in either group encouraged us to immunize two larger groups without selection as to Schick status. The first ("Group 3") received three doses of adsorbed diphtheria toxoid alone; the second ("Group 4") received three doses of adsorbed diphtheria toxoid, 1 Lf per dose, in adsorbed tetanus toxoid.

Methods

Subjects—Group 3: Five hundred and nineteen volunteer service school students were bled for antitoxin titration prior to the administration of two doses of 1 Lf adsorbed or precipitated purified diphtheria toxoid given three weeks apart. Five months later those men who were still available were bled again and were given another 1 Lf dose of toxoid. Three weeks later a final bleeding was taken. Two hundred and fifty-two men completed the entire study.

Group 4: Fifty-eight hundred and ten newly enlisted volunteer recruits received two doses (three weeks apart) of diphtheria toxoid, 1 Lf per dose, suspended in aluminum-phosphate adsorbed tetanus toxoid, in place of basic tetanus toxoid immunization. A special effort was made to learn of any reactions, ascribable to these inoculations, through close and sustained contact with the dispensary. In addition, 200 of these subjects were screened individually for possible untoward reactions. Two to three months later, from 187 of the men who were located on entering service school, interim blood samples were obtained for titration. Five months after the second dose—toward the end of the service school period—another bleeding was obtained and a third dose of the combined toxoids administered. Each man was examined the following day for local or systemic reactions possibly related to the inoculation. Three weeks later a final bleeding was taken on 170 subjects.

Toxoids—Toxoid Lot AK21, prepared at the Massachusetts Institute of Laboratories,[9] was used in about five-sixths of the subjects of Study 1. It contained 2 Lf purified diphtheria toxoid per ml. (1 Lf per dose) and 0.66 mg. Al (as phosphate) per dose as carrier. One-sixth of the subjects in Study 1 received an alum-precipitated toxoid prepared by Lederle Laboratories Division, American Cyanamid Company, from diphtheria toxoid purified by Dr. Louis Pillemer of Western Reserve University.[10] It likewise contained 1 Lf diphtheria toxoid per 0.5 ml. dose and

Table 2

Immunity Status	Massachusetts Toxoid (212 Men) Bleeding 1	2	3	Lederle Toxoid (44 Men) Bleeding 1	2	3
	Per cent			Per cent		
Susceptible (<0.003 u/ml.)	41.0	8.5	0.0	43	14	2
Borderline immune (>0.003, <0.03 u/ml.)	26.4	9.4	0.9	27	5	0
Solidly immune (>0.03 u/ml.)	32.6	82.1	99.1	30	82	98

was similar in principle to the Massachusetts preparation. Analysis of the antitoxin responses to the two toxoids showed no significant difference (Table 2).

The subjects in Study 2 received 0.5 ml. doses of combined tetanus and diphtheria toxoids Lot LK5A prepared at the Institute of Laboratories. It contained 1 Lf diphtheria toxoid, 5 Lf tetanus toxoid, and 0.66 mg. Al per dose.

Antitoxin Titrations — Antitoxin titers * were determined on venous blood by the rabbit intracutaneous method of Fraser.[11] Titrations were carried out at the levels of 0.001, 0.003, 0.01, 0.03, 0.1, 0.3 unit, and 1.0, 3.0, and 10.0 units. For the purpose of averaging titers, the geometric mean value was taken between the highest level at which no reaction was observed and the next higher level of titration. Values of 0.001 unit were arbitrarily regarded as lying at the mean between this level and 0.0003 unit.

Results

Group 3—Diphtheria Toxoid: The pre- and postinoculation immunity status of the subjects in this study is presented in detail in Table 3 and is summarized in Table 4.

The antitoxin levels observed have been grouped in Table 4 into three categories: (1) Those below 0.003 unit/ml. who are classed as "susceptibles" in accordance with the generally accepted finding that the threshold of the negative Schick test is at this general level of serum antitoxin; (2) those between 0.003 u/ml. and 0.03 u/ml. who may be regarded as having immunity of a probably transitory or borderline character; and (3) those with titers over 0.03 u/ml. who thus have at least 10 times the antitoxin level customarily associated with immunity and hence are likely to be protected even though their antitoxin level may fall 80 per cent or more—a fall which could readily occur in five years.[12]

It is apparent that the immunity status of the group prior to inoculation is not unlike that reported elsewhere (e.g.,[2] in which 40 per cent Schick-positives were found in 2,700 soldiers) and resembles the findings of an earlier phase of the studies here reported in which 190, or 37 per cent, of 511 naval service school personnel were Schick-positive. It is also evident from Table 3 that the 252 subjects who completed

* Antitoxin titrations were performed by Miss Vasilike Belios and Mrs. Rosamond Formal.

Table 3—Response to Three Doses of 1 Lf of Adsorbed Diphtheria Toxoid: Titers in 252 Subjects Prior to First Dose, and Before and After Third Dose—Group 3

Preinoculation Number	Preinoculation Titer	Postinoculation Titers <0.001	>0.001 <0.003	>0.003 <0.01	>0.01 <0.03	>0.03 <0.1	>0.1 <0.3	>0.3 <1.0	>1.0 <3.0	>3.0 <10.0	10.0 30.0	Geometric Mean Titer
87	<0.001	17 *	5	2	10	13	19	17	3	1	0	0.04
		1	0	0	1	3	29	31	19	3	0	0.4
16	>0.001 < 0.003	1	0	0	4	0	2	6	3	0	0	0.16
						1	2	6	5	2	0	0.8
16	>0.003 < 0.01				1	2	3	6	3	1	0	0.39
							3	6	5	2	0	0.86
51	>0.01 < 0.03				3	6	9	22	9	2	0	0.38
						2	5	21	15	8	0	0.94
26	>0.03 < 0.1						1	13	8	4	0	1.08
								9	9	8	0	1.70
27	>0.1 < 0.3						2	11	11	2	1	1.11
								6	13	7	1	2.02
23	>0.3 < 1.0							8	12	2	1	1.46
								4	14	4	1	1.95
5	>1.0 < 3.0								4	1	0	2.24
									3	2	0	2.82
1	>3.0 <10.0									1	0	..
										1	0	..
252	.. ..	18	5	2	18	21	36	83	53	14	2	
		1	0	0	1	6	39	83	83	37	2	

* Numerals in upper left portion of each box represent numbers of subjects with indicated postinoculation titer just prior to third dose; numerals in lower right represent numbers in each category 3 weeks after third dose.

Table 4—Response to Two and to Three 1-Lf Doses of Adsorbed Diphtheria Toxoid—Group 3

Immunity Status	Preinoculation Original Group	Preinoculation Final Group	Before Third Dose (Final Group)	After Third Dose (Final Group)
Susceptible (<0.003 u/ml.)	210	103	23	1
Borderline immune (>0.003, <0.03 u/ml.)	140	67	20	1
Solidly immune (>0.03 u/ml.)	169	82	209	250

the study were representative of the larger starting group.

Table 4 shows, in simple form, the effect of two and of three 1 Lf doses of aluminum-phosphate adsorbed diphtheria toxoid. The number of subjects who were solidly immune five months after the two basic doses of toxoid had risen from 32 per cent to 83 per cent. Three weeks after the third dose it had risen further to 99 per cent, an adequate immunity level by even the most rigid standards. Furthermore, no reactions came to the attention of the medical

staff of Great Lakes Naval Training Center following any of the diphtheria inoculations in this group. These findings suggested that the small doses of toxoid used, supported by the aluminum-phosphate carrier and given the advantage of long spacing before the third dose, provided an effective tool for mass immunization with minimal reactions.

Certain relationships of preinoculation levels to the response resulting from two and three 1 Lf doses of toxoid are to be found in Table 3 and are more clearly brought out in Figure 2:

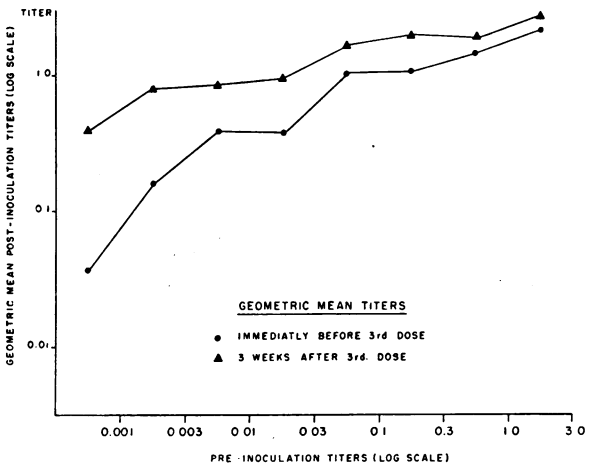

Figure 2—Relationship of postinoculation titers to preinoculation titers following two and three 1-Lf doses of adsorbed diphtheria toxoid

1. The relative increase in antitoxin level after inoculation of either two or three doses is in inverse proportion to the preinoculation level; i.e., the subjects with the lowest titers showed, in general, the greatest proportionate increase.

2. The relative increase in titer is greater with the first two doses than with the third.

3. The titers after three doses are grouped much more closely than after two doses. The geometric mean titers five months after two doses ranged from 0.04 unit in the group which started with < 0.001, to 2.24 units in the small group starting with > 1.0, < 3.0 units; this range is about seven times as great as is seen in the postbooster groups. Stated differently, the logarithmic standard deviation of the prebooster titers is 1.05, that of the postbooster titers is 0.58.

Group 4—Combined Tetanus and Diphtheria Toxoids: Preinoculation titrations were not performed on this group, since the number of men receiving doses one and two was necessarily very large in order to insure the finding of enough men later in the service schools to complete the study. Table 5 shows the distribution of immunity status of the service school group in this study at two intervals after the basic inoculations and three weeks after the booster dose. Although the number of susceptibles two to three months after the basic inoculations is surprisingly small, the distribution at the second (five-month) bleeding indicates that a not unexpected drift toward susceptibility has occurred at each level. The

Table 5—Response to Two and to Three 1-Lf Doses of Adsorbed Diphtheria Toxoid—Group 4

Immunity Status	Bleeding 1	2	3
Susceptible (<0.003 u/ml.)	2	26	2
Borderline immune (>0.003, <0.03 u/ml.)	40	21	5
Solidly immune (>0.03 u/ml.)	145	124	163
Total	187	171	170

Subjects received 2 doses of combined tetanus-diphtheria toxoid (1 Lf diphtheria toxoid per dose) 3–5 weeks apart, and a third dose 5 months later.
Bleeding 1 was taken 2–3 months after second dose.
Bleeding 2 was drawn 5 months after second dose, just before the third dose of toxoid.
Bleeding 3 was drawn 3 weeks after third dose.

third dose, however, brings the group immunity level up to a range comparable to that observed in Group 3.

Reactions were sought with special care after the third dose, since the writers, and others, have gained the impression that repeated inoculations are accompanied by an increased frequency of reaction with many immunizing agents, toxoids included.* There were no systemic reactions. About one-third of the men showed visible, but slight, swelling or erythema, or both, not sufficient to interfere with either duty or recreational activities. Four experienced moderate to severe local pain and swelling; three of these were hospitalized for one day. Three of these four reactions of consequence occurred among 49 men at the beginning of the third course of inoculations when the intramuscular route was being used. The subcutaneous route was employed thereafter for the remaining 125 men whose reactions were observed; only one marked reaction occurred thereafter.

Discussion

The findings here reported indicate that small doses of precipitated diphtheria toxoid, in the range of 1 Lf per dose, can be used successfully in the immunization of young adult Americans without regard to the selection of Schick-positives and without the occurrence of more than a very occasional side reaction. Furthermore, it appears that such small doses of diphtheria toxoid can readily and effectively be incorporated into precipitated tetanus toxoid. The use of diphtheria toxoid in this fashion, in order to establish a high general level of immunity in adult groups, affords a very great simplification over the currently employed procedures for adult immunization against diphtheria. For example, the present policy for diphtheria immunization of the U. S. Armed Forces is to Schick test the personnel concerned; administer 0.1 ml. of toxoid to the Schick-positives; administer 0.5 ml. two days later to nonreactors; and follow up with two doses of 1 ml. each at three- to four-week intervals, always dropping out reactors as they turn up following any dose. Such a schedule is complicated to administer; requires participants skilled in intracutaneous inoculation; and results in a by no means insignificant incidence of untoward reactions.

Inclusion of the small diphtheria component in tetanus toxoid greatly

* This impression has been clearly supported by the findings of Williams and Ellingson who have carried out a similar study in Air Force personnel.[13] It had also been noted by others previously.[14]

simplifies the immunization procedures for control of these two infections, reducing the total number of injections in a given group by more than half. The feasibility of using such a combination on a large scale in American adults was forecast by the success of the Canadian Armed Forces during the last five years in combining typhoid-paratyphoid vaccine with tetanus toxoid and small amounts of diphtheria toxoid.[4, 5] Because available data indicate that Canadians, on the average, are less toxoid-sensitive than Americans, however, it has been felt necessary to demonstrate the innocuousness of the combination for American adults. This has now been done, not only in the 170 men of Group 4, described here, but in much larger groups in the Army and Air Force who have received this combination in the course of routine immunization during field trials in 1953.[13, 15] The National Institutes of Health, U. S. Public Health Service, have established minimum requirements for the combined preparation which is officially designated as "Tetanus and Diphtheria Toxoids, Combined, Precipitated, for Adult Use."[16] *

In order to achieve the desired results with such a preparation, it is considered that certain principles must be followed, viz:

1. The diphtheria component must be of maximal antigenicity and fairly highly purified in order to insure adequate immunogenic response with minimal reactivity.

2. The toxoid must be precipitated or adsorbed to achieve an adequate immunogenic result.

3. The injection schedule must include not only the conventional primary immunization (two doses three to four weeks apart in the case of a precipitated preparation), but a third dose from six to 12 months later as well. It will be noticed that the term "booster" for this dose has been avoided throughout this paper. This concept has long been an integral part of the Danish diphtheria immunization programs in which the third dose is regarded as an essential component of basic immunization. Dr. Inga Scheibel's published data,[3] supported by additional unpublished findings,[6a] strongly support this principle, as do the findings presented in this report.

An adequate immunity level in at least 85–90 per cent of the subjects inoculated —a level attainable under the conditions outlined above—should be sufficient to prevent diphtheria from establishing more than an occasional individual infection in the group. Furthermore, the inclusion of a small amount of diphtheria toxoid in routine tetanus toxoid preparations would provide an effective basis for subsequent maintenance of the diphtheria immunity thus established, since current practice calls for periodic booster doses of tetanus toxoid in order to maintain immunity against lockjaw.

The data given in Tables 4 and 5 and shown graphically in Figure 2 support the well known observation that successive doses of an antigen tend to narrow the range of antibody levels in the subjects under study and to bring all subjects closer to a "plateau" somewhere under the ceiling of the antibody response. This effect has obvious practical significance in immunization programs, but it is also of theoretical interest. The term plateau is used here loosely, as must be the case in any immunological situation where 100-fold differences are normally to be expected. The appearance of what may—within these bounds—be called a plateau suggests that the subjects in question tended to approach the maximal response under the circumstances of the study. Whether this maximum range could have been significantly exceeded by increasing the dose can only be determined by further investigation.

* This product is being adopted for routine use in the U. S. Armed Forces beginning in 1955.

Summary

The studies presented indicate that the use of repeated and properly spaced small doses of diphtheria toxoid in the adsorbed state will induce a high level of immunity in unselected groups of young American adults with a minimum of side reactions. The findings of Danish and Canadian workers in this respect have thus been independently confirmed.

The application of this principle in adults, by combination of small amounts of diphtheria toxoid with other immunizing agents (e.g., tetanus toxoid) appears practical and useful. The combination of tetanus toxoid with small amounts of diphtheria toxoid, as described in this study, permits effective immunization of adults against both conditions with less than one-half the number of injections currently required.

REFERENCES

1. Edsall, G. Immunization of Adults Against Diphtheria and Tetanus. A.J.P.H. 42:393–400 (Apr.), 1952.
2. Pappenheimer, A. M., Jr.; Edsall, G.; Lawrence, H. S.; and Banton, H. J. A Study of Reactions Following Administration of Crude and Purified Diphtheria Toxoid in an Adult Population. Am. J. Hyg. 52:353–370, 1950.
3. Scheibel, I., and Tulinius, S. Immunization of Adults Against Diphtheria. Acta path. et microbiol. Scandinav. 27:69–77, 1950.
4. Sellers, A. H.; Caldbick, G. D.; Fraser, D. T.; Brown, M. H.; and Moloney, P. J. The Use of a Combined Antigen—TABTD. Canad. J. Pub. Health 41:141–145, 1950.
5. Sellers, A. H.; Caldbick, G. D.; Waters, G. G.; and MacQuarrie, M. J. Further Studies on the Use of a Combined Antigen—TABTD. Canad. J. Pub. Health, 42:269–274, 1951.
6. (a) Scheibel, I. Personal communication.
   (b) Fraser, D. T. Personal communication.
7. Edsall, G.; Banton, H. J.; and Wheeler, R. E. The Antigenicity of Single, Graded Doses of Purified Diphtheria Toxoid in Man. Am. J. Hyg. 53:283–295, 1951.
8. Altman, J. S., and Edsall, G. Response to Second Schick Test in Individuals Remaining Positive After One Schick Test. Federation Proc. 10:410, 1951.
9. Levine, L.; Wyman, L.; and Edsall, G. Chemical and Immunological Properties of Diphtheria Toxoid Purified by Ammonium Sulfate Fractionation. J. Immunol. 63:219, 1949.
10. Pillemer, L.; Wittler, R. G.; Clapp, F. L.; and Adam, J. N., Jr. Immunochemistry of Toxins and Toxoids. IV. Preparation of Purified Diphtheria Toxoid for Clinical Use. J. Immunol. 58:223, 1948.
11. Fraser, D. T., and Wigham, H. E. The Use of Rabbits for Intracutaneous Virulence Tests of B. diphtheriae, or Titration of Diphtheria Antitoxin: Preliminary Note. J.A.M.A. 82:1114–1115, 1924.
12. Fraser, D. T. Diphtheria Toxoid: A Review. Canad. Pub. Health J. 30:469, 1939.
13. Williams, Jack J., and Ellingson, H. V. Unpublished report.
14. Wishart, Franklyn O., and Reid, Marie Rose. A Study of the Diphtheria Antitoxin Response to Recall Doses of Specific Antigen. Canad. J. Pub. Health 38:131–140, 1947.
15. Long, A. P. Personal communication.
16. National Institutes of Health: Minimum Requirements for Tetanus and Diphtheria Toxoids Combined Precipitated, Adsorbed (For Adult Use). August 25, 1953.

A Chinese Maxim for Health Education

Dr. John Burton [1] cites the following Chinese maxim, which should prove a valid text for the most modern talk on health education:

If I hear it I forget
If I see it I remember
If I do it I know

[1] "Methods and Media in Health Education" by Dr. John Burton presented at the first meeting of The Expert Committee (WHO), Paris, December 1953—WHO, Division of Environmental Sanitation, Vol. 2, No. 7, Geneva, July, 1954.