

Constitutional Studies on Alcohol Tolerance by Intracutaneous Tests*

Ichiro TSUKUE

Department of Psyconeurology, Hiroshima University Medical School

(Director: Professor Masuho KONUMA)

Senogawa Mental Hospital (Director: Ichiro TSUKUE)

Takao SHŌHŌJI

The Institute of Environmental Medicine New York University Medical Center

アルコール研究 第2巻 第3・4号 (昭和42年12月刊行) 別刷
Separate-print from Vol. 2 No.3・4 of Japan. J. Stud. Alcohol
December 1967

Japan. J. Stud. Alcohol
アルコール研究

Constitutional Studies on Alcohol Tolerance by Intracutaneous Tests*

Ichiro TSUKUE

Department of Psyconeurology, Hiroshima University Medical School

(Director: Professor Masuho KONUMA)

Senogawa Mental Hospital (Director: Ichiro TSUKUE)

Takao SHŌHŌJI

The Institute of Environmental Medicine New York University Medical Center

(Received for publication Dec. 12, 1967)

Preface

There have been reports on the psychosomatic reactions to alcoholic beverages which can be observed objectively, and many have gone as far as pointing to hereditary traits, but it is felt that the understanding of the problem is essentially very vague.

Further, it has been reported that women and children have an extremely low tolerance to alcoholic beverages and that the tolerance in men decreases after passing middle age. These phenomena have been treated as constitutional or social problems indicating that here also the situation is vague.

In this report the author has set aside the pursuit of the hereditary traits from the mental disease point of view, and will take up the problem of alcoholic tolerance of the normal individual. At this present age when so many people drink alcoholic beverages, particularly when so many women have taken to drinking, it is felt the clarifying of such constitutional problems may lead to a new means toward grasping the true state of alcoholics and alcoholic intolerables, and may also play an important role in psychosomatic medicine, mental hygiene and sociology as has been stated in reports of similar work by others.

As one aspect of this study is to determine the true state of alcohol tolerance, antigenic experiments were performed, but this report will deal almost exclusively with the constitutional point of view.

There are, of course, various theories on whether the problem of alcohol tolerance can be clarified by antigenic studies. To cite an example, Harold E. Himwich¹⁾ expressed the opinion that alcoholism was felt to be an allergic hypersensitivity to alcohol or substance used in the distilling of alcohol, but these are not strongly supported by the current knowledge on immunology. Robinson and Voegtlin²⁾ claim that alcohol does not exert such

* The summary of this report will be presented at the 28th International Congress on Alcohol and Alcoholism to be held in Washington D. C. in September 15~20, 1968.

actions.

However, Takase⁸⁾ states that although a systematic study has not yet been conducted, when persons who show alcoholic intolerance receive intracutaneous injections of Allergen of 'sake', an allergen solution prepared by Torii Pharmaceutical Co. from 'sake' (Japanese rice wine), they tend to develop stronger erythema and eruptions of rather larger diameter than the normal individuals, which suggests the presence of an allergic reaction.

It is of importance to determine by systematic study as to whether or not this is an allergic reaction, but detailed review of the situation will be left for the future. In this paper only a review of bibliography dealing with this subject will be made. Efforts in this study were devoted chiefly to the pursuit of alcohol tolerance from the lateral point of view, and as an approach from the standpoint of preventive medicine to determine the type of constitution that would lead to the future development of alcoholism, the results of the skin tests alone were used to study the matter.

As one approach toward the establishment of predictions for each of the various samples, application of the theory of discriminant function was attempted.

I. Test Method

1) Test solution used was Sake-gen

Allergen of 'sake' (Torii Pharmaceutical Co.) is available on the market as an allergen extract used widely clinically as an effective means of diagnosis for allergic diseases. This is prepared by dialyzing refined 'sake' which is diluted to 1:1000 of its original weight with physiological saline into which 0.5% of phenol has been added, and then subjected to aseptic filtration. In other words, since it is a wasteful process to make an extract from 'sake', this solution is prepared by merely removing the low molecules from 'sake' per se. That is, when a cellophane film is used in dialysis, only the high molecule hetero-proteins remain (a type of residue), while the low molecules such as alcohol are removed. In tests using allergen of 'sake', this means that only the individual reaction to this residue is checked and the reaction to alcohol is not confirmed. As the use of such a test solution would not serve our purpose, a test solution termed "Sake-gen" which contains almost all ingredients such as alcohol, seasoning and antiseptics contained in refined 'sake' was newly prepared. This was used for the skin tests of individuals. In other words, second grade refined 'sake' (Brand name Sempuku with an alcohol content of 15.0~15.9 degrees) was made aseptic by filtering through a Zeiss filter. This had a pH of 4.3 and was used as Solution I. As Allergen of 'sake' has a pH of 6.6, in order to match this 26.5 ml of NaOH 1/10N was added to 200 ml of refined 'sake' and neutralized to a pH of 6.7. This was used as Solution II, and was sealed in brown 5 ml ampules together with nitrogen gas for storage and use when needed. Analysis by gas chromatography of the components of the refined 'sake' used is as shown in Table I.

Table 1.

Apparatus : Shimadzu Gas Chromatograph GC-1B Type (with hydrogen flame ionization detector)

Sample : Senpuku [A kind of the chief beverage (made from rice) of the Japanese] 2nd. Grade : $1\mu\text{l}$

Conditions: Range : 0.1

Liquid Phase: PEG-1500/Shimalite (20%)

Column Length: 3 m

Column Diameter: 4 mm

Column Temperature: 80°C

Detector Temperature: 100°C

Injector Temperature: 135°C

N_2 Gas Flow Rate: 80 ml/min.

Air Flow Rate: 0.9 l/min.

H_2 Flow Rate: 55 ml/min.

Retention Times of Peaks

Peak No.	A	B	C	D	E	F	G	H
Retn. Time (min.)	1.20	1.94	3.92	4.91	6.01	6.61	7.85	12.08

Peak No.	I	J	K	L	M	N	O
Retn. Time (min.)	14.42	19.17	19.48	24.32	28.00	36.9	40.66

Identification and Quantitative Analysis of Each Peak

All peaks were identified by adding of the anticipated components, and quantitative analysis was performed by peak area method.

Result

Peak No.	Compound	Peak Area (g) (with tracing paper)	Ratio of Peak
A	Air	—	—
B	Acetaldehyde	0.0097	1
C	?	0.0158	1.63
D	n-Butyl aldehyde or Ethyl acetate	0.0122	1.26
E	tert-Butanol	0.0143	1.47
F	Methanol	0.0067	0.69
G	Ethanol*	—	—
H	iso-Propanol	trace	—
I	n-Propanol	0.0664	6.85
J	iso-Butanol	0.0149	1.54
K	?	0.0297	3.06
L	?	trace	—
M	n-Butanol	trace	—
N	?	trace	—
O	Amyl alcohol	0.0652	6.72

Acidity: 1.40 ml.

Acidity shows the titrated volume of 0.1N-NaOH ml/10ml.

2) Method of performing skin test

Prior to performing the skin tests, a questionnaire as shown in Table II was taken by a psychiatrist. The test was performed at a room temperature maintained at 20–22°C and between 10 a.m. to 3 p.m. of the day.

Five persons each were admitted to the room and intracutaneous injections were administered at the same time. The precautions for injections were similar to those in general and 0.05 ml of the following 4 solutions, physiological saline, Allergen of 'sake', Sake-gen and Histamine 10⁻⁴, were administered from proximal to distal sites at several cm intervals on the left forearm by tuberculin needles. The needles were sterilized by dry heat method and changed for each injection.

Particular attention was paid to sterilization of the sites prior to injections. According to "Absorption of alcohol" by N. Harger et al.,⁴⁾ alcohol penetration through human skin has been reported. Further, Muehlberger made analysis of venous blood taken by a needle

Table 2. Date of Examination (Time, Humidity)

1. Name
2. Job description
3. Age (Date of birth)
4. Date of onset
5. Character
Nervous, worrisome, sociable, non-sociable, egoistic, timid, harmonious, neat, thorough, lonely, suggestibility
6. Skin
Rugged, average, sensitive, urticaria, drug allergy, asthma, easily develop rashes, eczema, easily develop severe sunburns
7. Maximum intake of alcoholic beverages (in terms of 'sake')
Do you drink regularly at supper? (If yes, since what age and how much?)
8. After you drink, do you become (red, not too red, pale)?
9. After you drink, do you become (gay, sleepy, violent, others)?
(No. of times hospitalized)
10. Others
Did you take a cold preparation last night?
Did you drink last night? (If yes, how much?)

Patient No.		5 min.	10 min.	15 min.	20 min.	30 min.
Physiological Saline	Eruptions					
	Erythema					
Allergen of 'sake'	Eruptions					
	Erythema					
Sake-gen	Eruptions					
	Erythema					
Histamine	Eruptions					
	Erythema					

administered through the skin which had been swabbed with alcohol, and reported that alcohol was present in the blood. Thus, as it has been carefully pointed out that when the blood is taken from the veins for blood analysis sterilizing of the skin with alcohol should be avoided, this author naturally did not use an alcohol swab prior to injections.

Until 20 minutes after intracutaneous injections the maximum and minimum diameters in mm of erythema and eruptions were checked every 5 minutes, and finally at 30 minutes. These values were averaged and used as basic data. (See lower portion of Table 2).

II. Test Results (Comparative Studies on Constitution)

1) Comparison of normal males and females

Sample composition

The above skin tests were carried out on 94 normal males and 81 normal females. The sample composition is as shown in Table 3.

Table 3. Distribution of Sample

Sex	Age 16-20	21-30	31-40	41-50	51-60	61-70	Total
Normal Male	54	20	5	2	0	0	81
Normal Female	10	57	13	8	6	0	94
Alcoholics	0	2	8	3	3	2	18

Further, 18 chronic alcoholics who had been admitted to this hospital were also subjected to similar tests. Comparisons with these patients will be presented later.

The sample composition for this report is not necessarily satisfactory, but it can not be helped as this study is yet in the preliminary stage.

The reactions to skin tests were pursued by time to predict those among the normal group who would move into the alcoholics group. Generally speaking, this type of data should be studied multidimensionally, but in this report they were handled one dimensionally. The results are as shown in Figures 1 to 8. That is, in order to observe the relationship of the mean diameters of eruption and erythema between the normal male and female groups over time for the respective test solutions, a 95% confidence interval was drawn. The line in the middle of the respective confidence intervals represents the mean values. Those of the alcoholic group are also shown, but as may be observed in the Figures, the respective groups all demonstrate their peculiar characteristics.

Ten determinations (5, 10, 15, 20 and 30 minute values for both eruptions and erythema) were made for each individual and each of the values were considered separately. The results of the test to determine if there is a difference between both groups are as shown in Table 4 excluding the rightmost column. Table 4 shows the results of statistical calculations based on a *t*' distribution to test the hypothesis that there is no difference between the males and females. (Q signifies eruptions and E erythema). Part of the determinations followed a typical logarithmic normal distribution.

As may be readily seen from Table 4, there is a significant difference between males and females for each determination of eruption caused by physiological saline, Allergen of 'sake', and Sake-gen I. On the other hand, determinations of erythema at 5 and 10 minutes

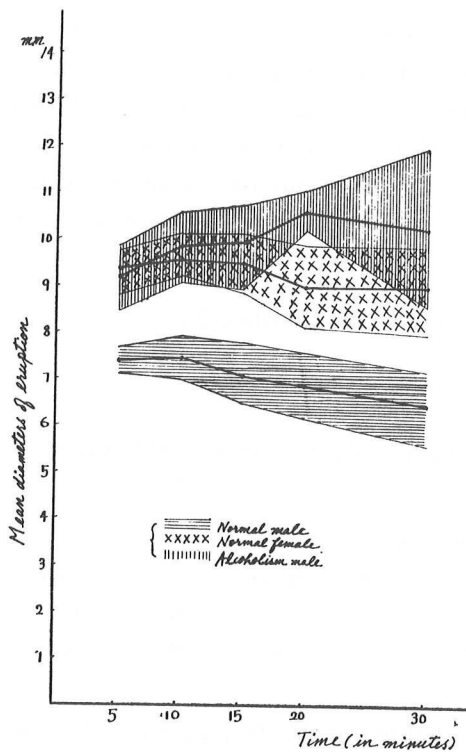


Fig. 1. 95% Confidence Interval of Eruption for a Physiological Solution of Sodium Chloride

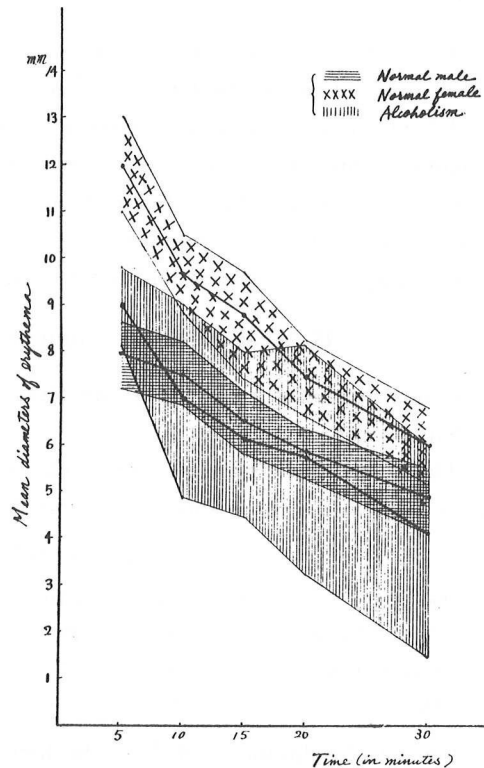


Fig. 2. 95% Confidence Interval of Erythema for a Physiological Solution of Sodium Chloride

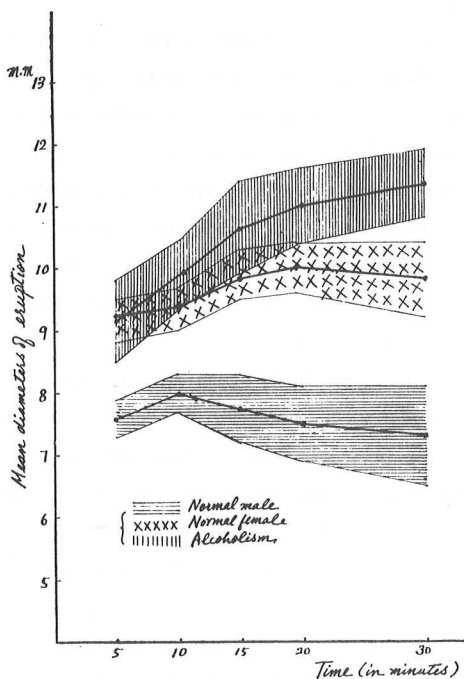


Fig. 3. 95% Confidence Interval of Eruption for Allergen of Sake (TORII)

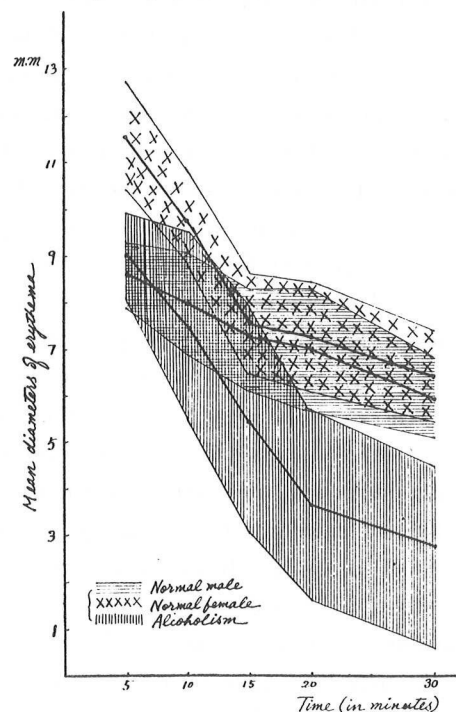


Fig. 4. 95% Confidence Interval of Erythema for Allergen of Sake (TORII)

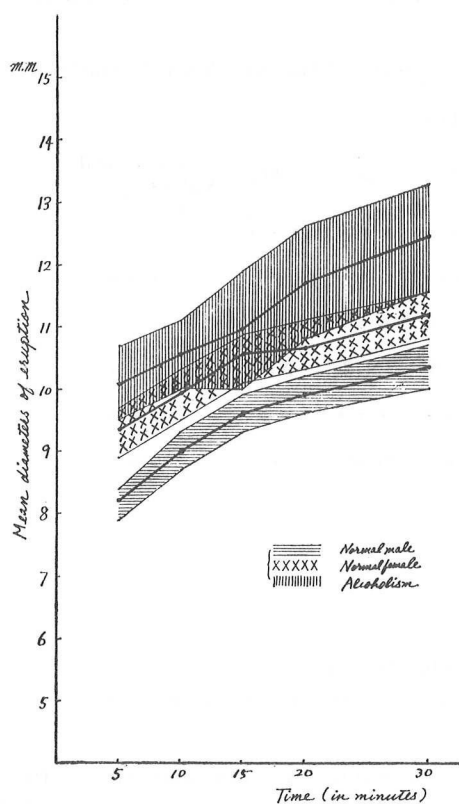


Fig. 5. 95% Confidence Interval of Eruption for Sake-gen I

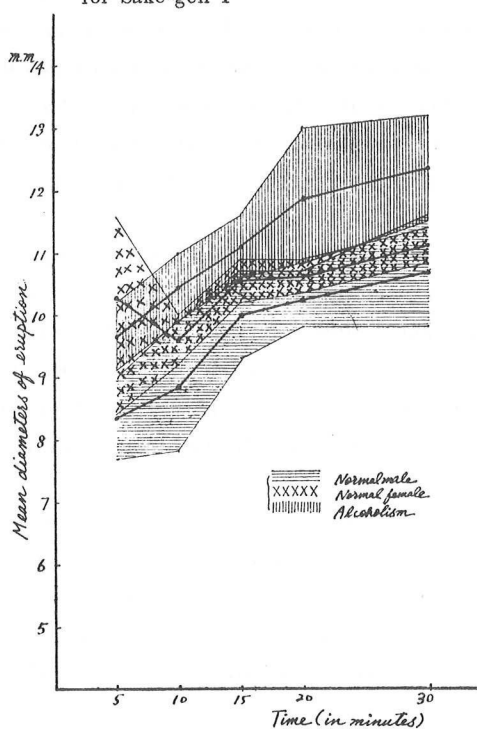


Fig. 7. 95% Confidence Interval of Eruption for Sake-gen II

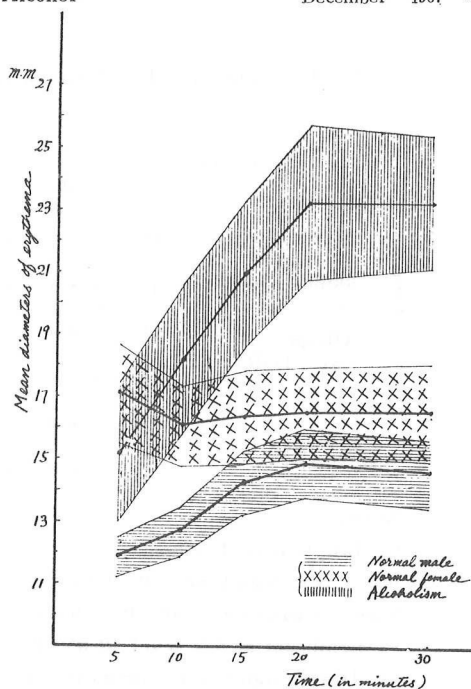


Fig. 6. 95% Confidence Interval of Erythema for Sake-gen I

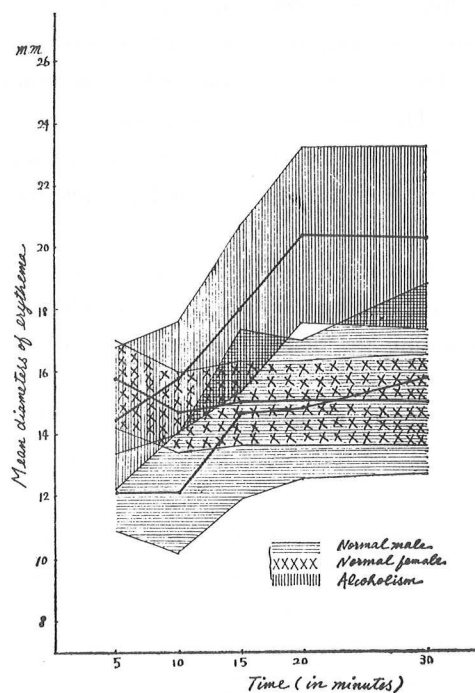


Fig. 8. 95% Confidence Interval of Erythema for Sake-gen II

Table 4. Significance Test between the Means of Normal Males and Normal Females

		Normal Males						
Normal Females	Time (minutes)		5	10	15	20	30	5-dimensional Values
	Allergen							
	Physiological solution of sodium chloride	Q	***	***	***	***	***	***
		E	***	***	**	*	*	***
	Allergen of sake (TORII)	Q	***	***	***	***	***	***
		E	***	*	N.S.	N.S.	N.S.	***
	Sake-gen I	Q	***	***	***	***	**	***
E		***	***	*	Sugg.	*	***	

Notation

Q=Eruption E=Erythema

N.S.: Non Significant ($P \geq 0.10$);Sugg.: Suggestive ($0.10 > P \geq 0.05$);*: Significant at 5% level ($0.05 > P \geq 0.01$);**: Highly significant; significant at 1% level ($0.01 > P \geq 0.001$);***: Very highly significant; significant at 0.1% level ($0.001 > P$); where P is the Probability of significant level.

after injection showed a significant difference between sexes, but thereafter the statistical difference seemed to gradually become less. This is believed to be due to the constitutional difference between males and females. It is felt that there is not any difference between sexes for both eruptions and erythema when Sake-gen II was used. It is believed that this is due to a change resulting from the fact that the test solution was neutralized with NaOH.

The above tests were made to determine whether or not there were any differences at the respective times of determination, however, the determinations at the respective time intervals do not indicate independent reaction values, but are naturally closely related to one another.

Generally, conclusions of the skin test are always based on the value read 15 minutes after the injection, but as it was felt that rather than attempting to debate differences while ignoring the determinations taken at the abovementioned time intervals, it would be better to consider the values at the respective time intervals. Therefore, each of the 5 determinations were considered as 1 set of multivariate values and Hotelling's T^2 statistics was used to test the difference between sexes. The results are as shown in the rightmost column of Table 4.

When tests for sex difference in erythema are made for each determination, it appears as if the difference gradually lessens with time, but when the differences in this column are treated as a multivariate value, it can be shown that there is still a markedly significant difference between the sexes. This indicates that there is a difference between sexes in the way that erythema changes. Naturally, there is a very close relationship between Table 4 and Figures 1-8.

As an example, the erythema of Sake-gen I shows a definite separation between normal

males and females up to 10 minutes after injection as may be observed from Figure 6, but thereafter the confidence intervals of both sexes show areas of overlapping.

Further, the erythema of Sake-gen I shown in Table 4 presents a significant difference at the 0.1% level for the 5 and 10 minute values, but the values after the 15 minute determination did not show such highly significant differences. This agrees with the situation described in Figure 6.

From the above, it has been demonstrated that the appearance of eruptions varies greatly between sexes, females showing greater reaction values than males. On the other hand, the appearance of erythema is greater among females than males up to 10 minutes after injection, but thereafter the difference gradually lessens.

2) Normal males and alcoholics

Introduced hereunder is the criteria for predicting from the skin test reaction results, normal males who will become alcoholics.

a) Study was made of the constitutional difference between normal males and alcoholics, and the results noted between the 2 are as shown in Table 5 and Figures 1-8.

As shown in Table 5, there is no statistical difference in the erythema values between

Table 5. Significance Test between the Means of Normal Males and Alcoholics

		Normal Males							
Alcoholic males	Time (minutes)		5	10	15	20	30	5-dimensional Variable	10-dimensional Variable
	Allergen								
	Physiological solution of sodium chloride	Q	***	***	***	***	***	***	
		E	N. S.	N. S.	N. S.	N. S.	N. S.	N. S.	
	Allergen of sake (TORII)	Q	***	***	***	***	***	***	} * * *
		E	N. S.	N. S.	N. S.	*	*	Sugg.	
	Sake-gen I	Q	***	***	**	***	***	***	} * * *
E		***	***	***	***	***	***		

normal males and alcoholics for physiological saline and Allergen of 'sake'. Thus, this implies that the erythema due to physiological saline can be used as a control when alcoholics and normal persons are discussed. That is, it could be used as an index to determine if there is any bias due to skin sensitivity, etc.

As in the case of Table 4, Table 5 also has a very close relationship with Figures 1-8.

Reactions to Sake-gen I (Figures 5 and 6) show little differences between sexes, but there appears to be a great difference between alcoholics and normal males. Thus, this indicates that the Sake-gen I test is a highly effective test for comparing alcoholics and normal males, and making predictions.

It is felt that if the abovementioned Sake-gen I results and the data on erythema following injection of physiological saline together with skin sensitivity are taken into consideration, it should enhance the possibility of distinguishing between alcoholics and normal males. That is, it is hoped it would become possible to predict who in the normal

male group would become an alcoholic.

b) As mentioned earlier, as a method for establishing this criteria, the discriminant analysis theory developed by R. A. Fisher in 1936 was applied. In this analysis, a linear function of observed values which would serve to better separate 2 or more groups was sought. In this report, the 2 groups of normal males and male alcoholics were the subjects of study.

In order to make a discriminant function, decision should be made as to what to select as the variables. Although not entirely satisfactory for our purposes, the data used in the study were the 30 variables of 5, 10, 15, 20 and 30 minute eruption and erythema values for physiological saline, Allergen of 'sake' and Sake-gen I. These determinations will be expressed by the following variables.

X_1 : 5 minute value of eruptions due to physiological saline

X_2 : 10 //

X_3 : 15 //

X_4 : 20 //

X_5 : 30 //

X_6 : 5 minute value of erythema due to physiological saline

X_7 : 10 //

X_8 : 15 //

X_9 : 20 //

X_{10} : 30 //

X_{11} : 5 minute value of eruptions due to Allergen of 'sake'

X_{12} : 10 //

X_{13} : 15 //

X_{14} : 20 //

X_{15} : 30 //

X_{16} : 5 minute value of erythema due to Allergen of 'sake'

X_{17} : 10 //

X_{18} : 15 //

X_{19} : 20 //

X_{20} : 30 //

X_{21} : 5 minute value of eruptions due to Sake-gen I

X_{22} : 10 //

X_{23} : 15 //

X_{24} : 20 //

X_{25} : 30 //

X_{26} : 5 minute value of erythema due to Sake-gen I

X_{27} : 10 //

X_{28} : 15 //

X_{29} : 20 //

X_{30} : 30 //

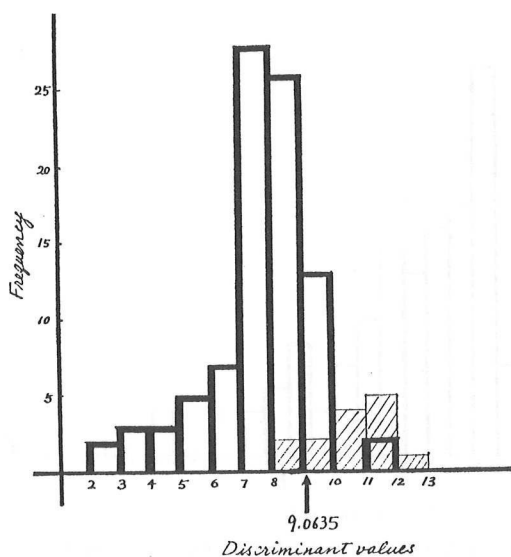


Fig. 9. Frequency Distribution of Discriminant Values for Eruption (5'~30') of a Physiological Solution of Sodium Chloride

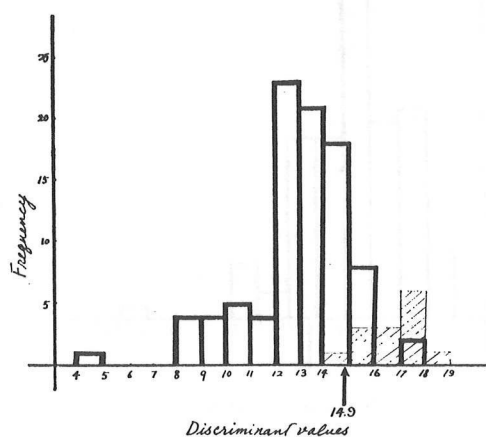


Fig. 10. Frequency Distribution of Discriminant Values for Eruption (5'~30') of Allergen of Sake (TORII)

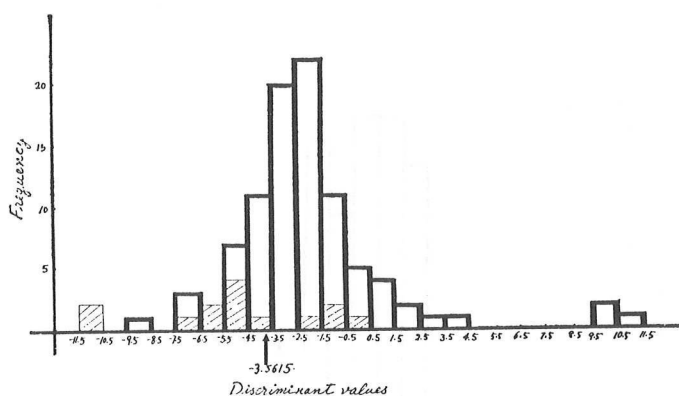


Fig. 11. Frequency Distribution of Discriminant Values for Erythema (5'~30') of Allergen of Sake (TORII)

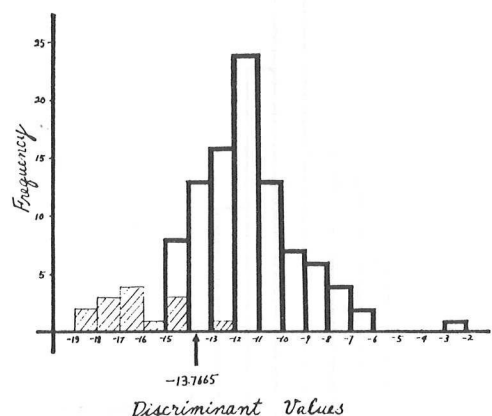


Fig. 12. Frequency Distribution of Discriminant Values for Erythema and Eruption of Allergen of Sake (TORII)

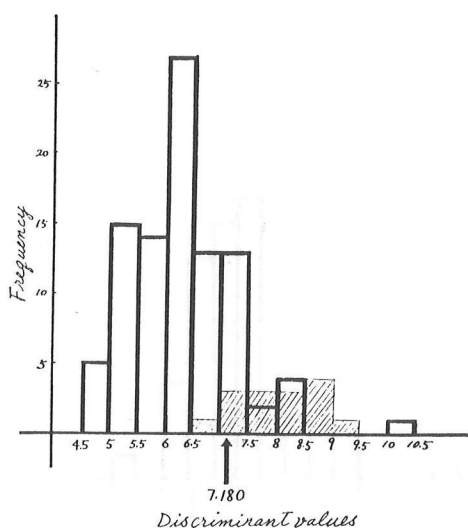


Fig. 13. Frequency Distribution of Discriminant Values for Eruption (5'~30') of Sake-gen I

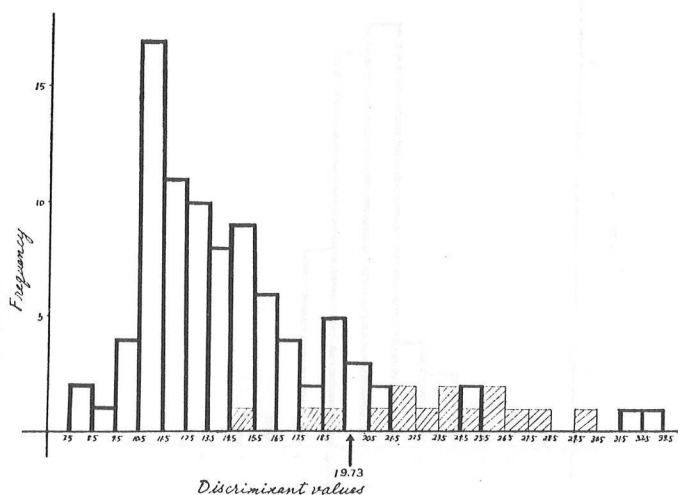


Fig. 14. Frequency Distribution of Discriminant Values for Erythema (5'~30') of Sake-gen I

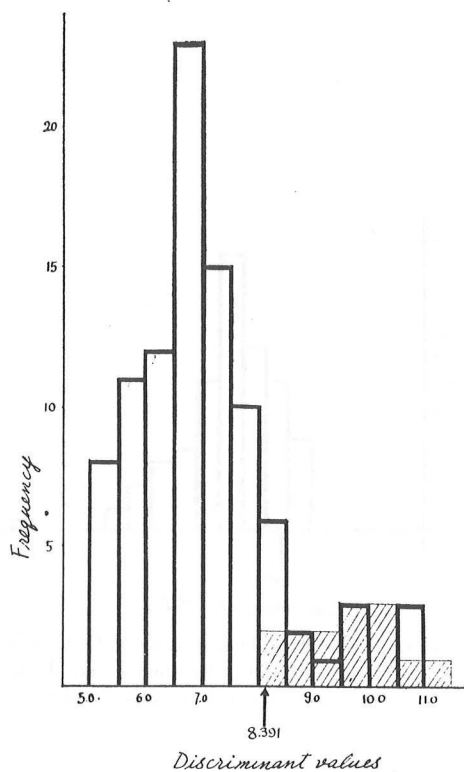


Fig. 15. Frequency Distribution of Discriminant Values for Erythema and Eruption (5'~30') of Sake-gen I

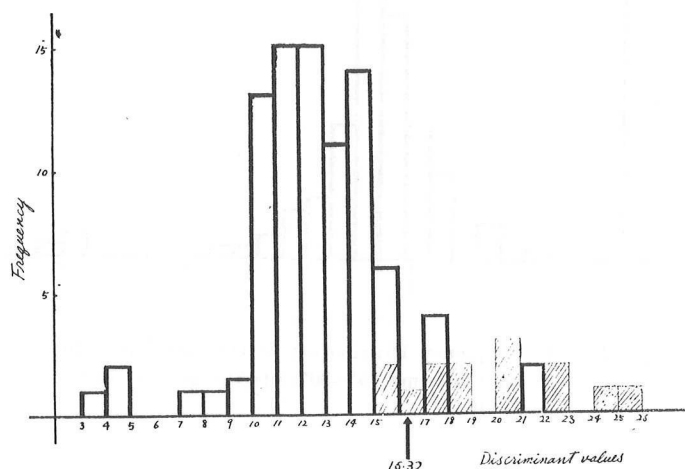


Fig. 16. Frequency Distribution of Discriminant Values for Erythema and Eruption Values at 15 Minutes of a Physiological Solution of Sodium Chloride Allergen of Sake (TORII) and Sake-gen I

In order to prepare a discriminant function using these variables to distinguish between normal males and alcoholics, it is necessary to seek the 30 coefficients c_i ($i=1,2,\dots,30$) among the linear functions of these variables

$$y = \sum_{i=1}^{30} c_i x_i$$

which best separate the 2 groups. The coefficients c_i ($i=1,2,\dots,30$) thus obtained are called coefficients of discriminant functions and y is called the discriminant value. When m_1 , m_2 are the mean discriminant values of the normal males and alcoholics respectively,

$$m = \frac{m_1 + m_2}{2}$$

is called the discriminant criteria value which best separates the 2 groups. When value m is assumed to be a selection from a normal population which provides a common variance for the 2 groups and the priori probability of both groups is the same, then the value will minimize the probability of making a mistake in prediction. That is, a value that keeps the overlap of the 2 groups at a minimum.

From Table 5, it was learned that there is an extremely significant difference between the mean vector of the mean diameter of the eruptions caused by physiological saline in the normal males and the alcoholics. The discriminant function for the 5 eruption diameters resulting from physiological saline injection is

$$y = 0.745X_1 + 0.293X_2 - 0.425X_3 + 0.421X_4 + 0.030X_5.$$

From this function the individual discriminant values were sought and presented in a histogram as shown in Figure 9. (The bold lines indicate the normal males while the thin lines represent the alcoholics. The discriminant criterion value is indicated by an arrow.) As may be seen from this graph, it appears difficult to distinguish the normal males from alcoholics based on eruptions of physiological saline alone.

The mean vector of eruptions resulting from Allergen of 'sake' showed a highly significant difference between the 2 groups, but the mean vector of erythema did not necessarily show a significant difference. The respective discriminant functions are as follows.

$$y = 0.595X_{11} + 0.779X_{12} + 0.060X_{13} + 0.164X_{14} + 0.093X_{15}$$

$$y = 0.581X_{16} - 0.093X_{17} - 0.373X_{18} + 0.670X_{19} + 0.256X_{20}$$

The individual discriminant values are obtained from the above, and when histograms are prepared they appear as shown in Figures 10 and 11.

However, the measurement values for eruptions and erythema are not to be used independently, thus when they are combined and a 10 dimensional vector is considered, there is still a highly significant difference between the 2 groups. Therefore, when a discriminant function based on 10 dimensional determinations is prepared it will be as follows.

$$y = -0.403X_{11} - 0.824X_{12} - 0.027X_{13} - 0.215X_{14} - 0.122X_{15} \\ - 0.087X_{16} + 0.021X_{17} - 0.106X_{18} + 0.272X_{19} + 0.056X_{20}$$

This is used to obtain the individual discriminant values, and when a histogram is prepared it appears as shown in Figure 12. In this case, the discriminant criterion value is -13.8 as shown by the arrow, and when an individual with a value lower than this is considered

to be an alcoholic, the probability for error would be about 10%.

Next, the discriminant function for eruptions due to Sake-gen I prepared by this author is as follows.

$$y=0.661X_{21}-0.003X_{22}-0.590X_{23}+0.289X_{24}+0.363X_{25}$$

The histogram for the discriminant values is as shown in Figure 13. When using the eruption values of Sake-gen I alone to distinguish between normal males and alcoholics, if those whose values were larger than the discriminant criterion value of 7.2 are considered alcoholics the probability for misclassification would be about 20%.

The discriminant function for erythema of Sake-gen I is as follows.

$$y=0.308X_{26}-0.091X_{27}-0.475X_{28}+0.530X_{29}+0.625X_{30}$$

The histogram is as shown in Figure 14, and the probability of misclassifying those with discriminant values greater than the discriminant criterion value of 19.7 (indicated by arrow) as alcoholics is about 20%. Further, then the discriminant values for erythema and eruption for Sake-gen I are combined, the function is as follows.

$$y=0.683X_{21}+0.061X_{22}-0.610X_{23}+0.272X_{24}+0.229X_{25} \\ +0.032X_{26}-0.018X_{27}-0.087X_{28}+0.141X_{29}+0.055X_{30}$$

The histogram corresponding to this is as shown in Figure 15. The discriminant criterion value of 8.4 is shown by an arrow. When the discriminant value is greater than the criterion value, the probability for misclassification is about 15%.

At present, since skin test results are generally determined by the 15 minute value, the discriminant values for eruptions and erythema of physiological saline, Allergen of 'sake' and Sake-gen are as shown in the histogram of Figure 16. In this case the discriminant value (shown by arrow) is 16.3 and when those with discriminant values greater than this are regarded as alcoholics, the probability for misclassification is about 25%.

Figure 17 shows a graph with the discriminant values of Allergen of 'sake' and Sake-gen plotted on the vertical and horizontal axes respectively. The dots indicate normal males and the Xs alcoholics. The upper left ellipse shows the 90% confidence ellipse for normal males while that in the lower right shows the same for alcoholics.

The equations for these 2 ellipses are as follows.

$$0.141(X-7.12)^2+0.127(X-7.12)(Y+11.420)+0.046(Y+11.420)^2=1$$

$$0.230(X-9.67)^2+0.068(X-9.67)(Y+16.11)+0.057(Y-16.11)^2=1$$

The meaning of the right lower ellipse is that of those who are within the ellipse, 9 out of 10 may be regarded as alcoholics. Further, those with extremely sensitive skin, alcohol intolerables and problem drinkers are felt to group together respectively. Such cases and the analysis on cases where histamine was used will be presented at a later date.

III. Results of Screening Test

1) The criteria for normal males and alcoholics were prepared and explained in 2) of II, and 361 industrial workers of a company were subjected to the same type of skin test mentioned above and an attempt was made to apply the said criteria to them. The age distribution of the sample is as shown in Table 6. Further, prior to the skin test, all

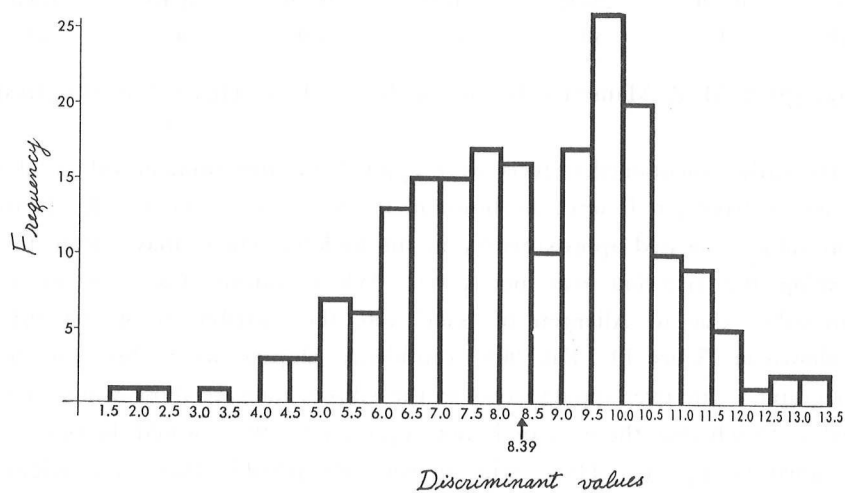


Fig. 18. Frequency Distribution of Discriminant Values for Erythema and Eruption (5'~30') of Sake-gen 1 (361 Normal Males)

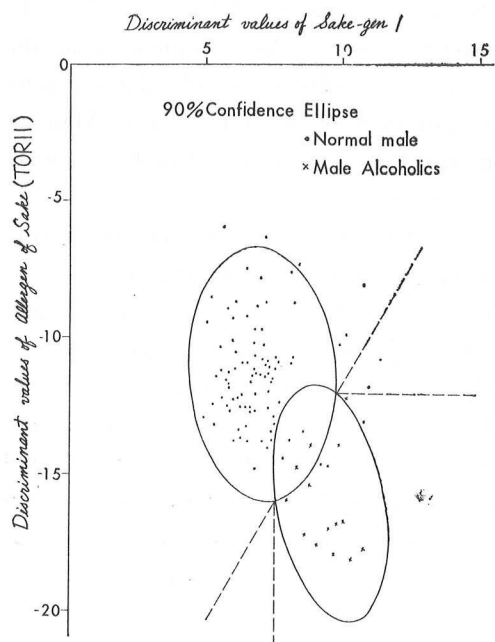


Fig. 17. 90% Confidence Ellipse

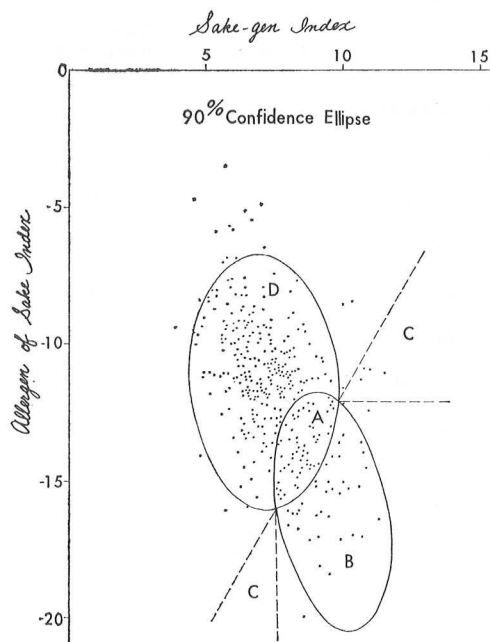


Fig. 19. 90% Confidence Ellipse

Table 6.

Age	16-20	21-30	31-40	41-50	51-60	Total
Male	1	40	126	158	36	361

workers were given M. P. Manson's Alcohol Addiction Test. This will be described later in this report.

When the earlier mentioned criteria were applied, the discriminant values of eruption and erythema for Sake-gen I, were as shown in the histogram of Figure 18. In this figure the criterion value is located approximately in the middle, which may mean the sample used to develop the criterion was not good. When consideration was given to the discriminant values due to Allergen of 'sake' too, the distribution of the 361 subjects became as shown in Figure 19. The 90% confidence ellipses were also entered on this distribution graph. The overlapping areas of the ellipses indicate those whom it could not be predicted as to whether there is, or is not, a possibility they would become alcoholics, and were called Group A. Those who it was felt possible that they might become alcoholics were made Group B, while those who were felt to be within normal limits were classified as Groups C and D. According to this classification, there were 30 in Group A, 70 in B and 261 in Groups C and D.

2) Alcohol Addiction Test ⁵⁾⁶⁾

As explained above, all 361 male industrial workers were given M. P. Manson's Test

Table 7. Distribution of A-Test Score

	Manson	Industrial workers in Japan
No. of subjects	61	361
Score		
55~59	0	0
50~54	0	0
45~49	0	0.3
40~44	0	1.1
35~39	0	2.2
30~34	0	8.0
25~29	0	12.2
20~24	0	26.3
15~19	4.9	20.2
10~14	13.1	16.6
5~9	27.8	11.1
0~4	54.2	2.0
%	100.0	100.0

before they received their skin test. The resulting distribution of the score is as shown in Table 7. As is evident from the Table, the results of the distribution were completely different from that of Manson, in that 181 or more than half had a score exceeding 20.

At the same time questions on drinking history, amount of intake, state of drunkenness (physical and mental), character and constitution were asked by a psychiatrist. The questions were the same as those of Table 2 of I, but particular attention was paid to items which are felt to be affected by the skin test such as forgetfulness when drunk and whether face becomes red or pale after drinking as well as a distribution by group of those with an allergic constitution (Table 8). However, there were little statistical relations between any of these physical conditions and Group A-B combined or Group C-D combined.

Table 8.

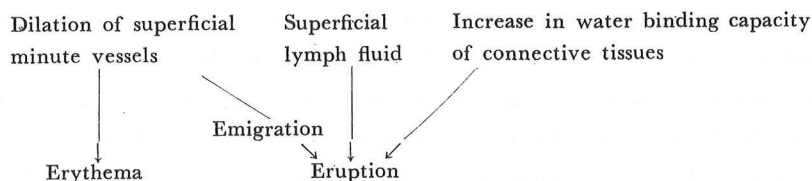
	A	B	C, D	Total
	30	70	261	361
Forgetfulness after drinking	7	16	45	68
Redness of face after drinking	13	37	120	170
Allergic constitution	2	6	26	34
Score of 20 and over for Alcadd. test	15	30	132	177

alcoholics were combined, they totalled 100, and of this number, there were 45 with scores of 20 or more points under Manson's Test.

Therefore, after completing the above analysis, the supervisors and coworkers of the above 45 were asked about the drinking habits and state of drunkenness, but as it involved a personal matter, there were many instances where definite information could not be obtained. Excluding the 15 on whom no information could be obtained, there were 13 (43%) who were considered problem drinkers, 8 with gastroduodenitis, 3 receiving treatment for diabetes and 1 with hypertension.

IV. Considerations

1) In his paper "Skin Reactions from the View-point of Skin Physiology", Professor Takase explains the model for the developmental mechanism of eruptions and erythema as follows.



Due to the effects of allergic and non-allergic mechanisms on the vascular, lymphatic and connective tissue systems, erythema, and eruptions which can hardly be distinguished from one another develop. The severity of the reactions vary depending on the condition of the individual, state of disease preparedness, local factors (such as site of injection, skin vessel differences due to temperature, light-particularly difference in exposure to ultraviolet light), age, such allergen factors as vessel dilators or permeability promoting agents contained in the allergen itself, and the pH.

Physiological saline is a foreign body being of different composition from blood, resembling it only in the fact that it is isotonic. Therefore, it presents an even greater foreign body reaction to the skin per se. Thus, when it is injected intracutaneously, it is only natural that erythema and eruptions develop according to the theory advocated by Professor Takase.

Next, Allergen of 'sake' has approximately the same pH as physiological saline, but 'sake' contains high molecular proteins, and even though small in quantity, the reaction

When those with a score of 20 or more by Manson's Test were considered problematic, there were as many as 181 who met this category as explained earlier, but when those in Group A for whom it could not be predicted as to whether there is or is not, a possibility they would become alcoholics and those of Group B for whom it was felt possible that they might become

toward this foreign body should be much greater than that against physiological saline. Thus, the development of erythema and eruptions were more severe than that for physiological saline as indicated earlier.

The Sake-gen originated by the author is different from Allergen of 'sake' as it retains the ingredients of 'sake' as well as the alcohol. The only minor difference is that the pH has not been supplemented. Therefore, it is of an even higher degree of a foreign body. From this point of view, 'Sake-gen II' in which the pH had been supplemented was prepared, but it is felt that this preparation would not play an important role, at least, in the prediction of alcoholics.

2) The product of TORII Pharmaceutical Co. known as Allergen of 'sake' was prepared under the assumption that the developmental mechanisms of erythema and eruptions are an antigen-antibody reaction, but it is actually merely a reaction to the injection of a foreign body, and whether it is an allergy or not will require further careful study.

A review of literature on studies on alcohol tolerance by use of antigen-antibody reaction was made. The author introduced in the preface various opinions on constitutional alcohol tolerance obtained from genetic and constitutional studies. There were opinions that the difference between the heavy drinkers and the intolerables is merely the degree of alcohol intake, while others claim that the two are traits which are distinctly separate. Whatever the case, the opinion is that the matter should be pursued from the constitutional point of view. However, the constitutional classifications are based on findings after alcohol had been drunk, and there has been no literature on objective patho-physiological studies such as immunological studies using alcohol as an allergen. Careful observation of the state of drunkenness presents many findings which suggest the presence of immunological reactions to alcohol. Thus, the author sought to pursue the problem by bibliographical review.

Urbach studied the skin reactions to alcohol, and reported that there were allergic substances in the trace of ingredients that remain after being combined into the beverage during the process of manufacturing, in addition to the major component, methyl alcohol. Such ingredients include barley, malt, beer yeast, rye, and wheat as well as egg white and fish gel used to clear the 'sake'. Further, he stated alcohol promotes the permeability of the gastric mucous membrane, causing such atypical actions as the development of allergy by readily absorbing into the blood foods, mainly proteins, which had not been sufficiently digested.

3) However, no further reports claiming the phenomenon of heavy drinking or alcohol intolerance to be an allergy have been found in Western countries nor Japan, but TORII Pharmaceutical Co. used the findings as a hint and put on the market Allergen of 'sake' (4 types, for beer, 'sake', 'shochu' (a low class distilled drink) and whiskey). As explained earlier this is not 'sake' per se, but consists of its high molecular proteins, and does not contain alcohol. There have been reports as shown below on experiences with its use.

Professor Takase reported that several persons who readily became red or break out in perspiration in the face, neck, chest and back shortly after drinking alcohol were selected and subjected to an Allergen of 'sake' test, and found that they showed tendencies of

developing severer erythema reactions and eruptions of slightly larger diameters than normal persons.

It was demonstrated that by the 'Sake-gen I' test, not only the state of drinking is demonstrated in the face, but also that it is accentuated depending upon the degree of sensitivity of the skin. Professor Takase is also carrying out studies using allergen solutions by infusion to the rabbit mensentric to determine such atypical effects as vessel dilation and hemorrhagic factors, but the results with the use of Allergen of 'sake' have been negative. However, he states it cannot be denied that specific allergens themselves or something else may contain the stimulant factor which strongly exaggerates the intracutaneous skin reaction.

However, even the work of Professor Takase has not been carried out systematically, but has been sporadic, and further it has not been work where the tendency of alcohol tolerance has been pursued pathophysiologically or immunologically.

Intracutaneous tests of 'sake', studies have been done by Dr. Tetsuo Kishi⁸⁾ and reported under the title of Experimental Pathological Studies on 'Sake'. He used 5 albino rabbits selecting the same 6 sites on the back for injection of 0.2ml of 'sake' every other day either subcutaneously or intracutaneously, and observed the histological reaction. According to the report, initially he noted psuedo-neutrophilic and monocytic infiltrations, but since similar reactions were noted when distilled water had been administered, these were not considered as peculiar. Nine days after injection, he noted clumps of plasmacyte appearance in the bifurcations of the arterioles throughout the whole course. However, this author has been unable to locate the original paper, and thus is not aware of the details of the course nor the methods, but immunological consideration was not provided.

4) 'Sake-gen' originated by the author, was not limited to determining the allergic reaction of high molecular hetero-proteins after the low molecules and the alcohol components contained in 'sake' had been removed as in the case of Allergen of 'sake', but in addition to possessing the requirements of Allergen of 'sake', it had converted the alcohol itself into a 'gen', which made its effects stronger and the results more natural. It is felt that alcohol intolerance is not regulated by the high molecular hetero-proteins alone, and that it is natural to consider that factors related to such should be sought from alcohol per se as well.

However, since the pH of 'Sake-gen I' is 4.3, together with the sensitivity of the skin and facial changes due to drinking, consideration must be given to the fact that there is a general tendency for the reaction value being accentuated. It is felt it will be necessary to adopt measures to counter this in the future.

Also the fact that some feel drunk after receiving the small dose of only 0.05ml intracutaneously is not merely a psychosomatic reaction, that is, rather than the explanation that although small in dose the knowledge that alcohol is being injected causes intoxication, it is felt such is due to the biological reaction to an allergic factor.

5) Although there is still room for study regarding the Sake-gen that the author used as a skin test solution, it is felt that using 'sake' to study the problem of alcohol tolerance is the most natural and reasonable approach,

The complex state of the reaction to this test solution and the problem of allergy is as described above. It is felt that many interpretations can yet be made, but even though the results obtained by the method the author used may seem simple and direct, they are a definite set of data and do serve to support some aspects of the research done to date. Further, they provide a basis for future development of studies and are strongly felt to be quite meaningful. However, it must be stated that the intracutaneous test is not an omnipotent method, but is merely one approach in trying to attain our goal.

From the fact that the results obtained by statistical processing show that physiological saline, Allergen of 'sake' and Sake-gen have demonstrated significant differences in the reaction values for eruptions and erythema between normal males and females, it is felt that the author was able to present data to support the general claim that the alcohol tolerance of females is less than that of males.

Further, it was noted that there was also a statistically significant difference between normal males and alcoholics, and the presence of a constitutional difference between the 2 groups was suggested. Further, when the skin test was repeated 10 times on the same individual, there were also considerable differences among the repeated test results. This implies that skin tests should not be performed just once, but should be repeated 2 or 3 times. Details of these findings will be presented in the author's next report.

On the basis of these differences, the author attempted to predict from among the normal males those who would eventually become alcoholics, which was the objective of this study. Also from the standpoint of preventive medicine, it was felt that the application of discriminant analysis⁹⁾¹⁰⁾ to grasp such a trend during the early stage would prove to make this method most useful.

This is based on the fact that the linear function method which would best distinguish between normal males and alcoholics (males) was sought and applied to tests on 361 industrial workers of a certain company, and the general conclusion was that this method was useful as a screening test.

Whatever the case, it was possible to demonstrate objectively by skin tests and statistical procedures that there are sex differences as well as constitutional differences in the psychosomatic reactions to alcohol. Further, by the use of discriminant function, it was felt it would be possible to suggest as to whether there was a possibility that a certain person would become an alcoholic in the future, and thus it was pointed out that from the preventive medicine viewpoint, particularly mental hygiene, as well as from the sociological viewpoint, the approach was highly useful.

From the broad point of view of constitutional studies, there are still many shortcomings, however, it is felt that this study will serve as a foothold, and it is the author's intention to make deeper probes so as to be able to grasp the essence of this constitutional problem.

V. Conclusion

1. The method for making Sake-gen, an origination of the author, is explained, criticism toward its application was made on the basis of bibliographically review, and its usefulness was discussed,

2. Ninety-four normal males and 81 normal females, and 94 normal males and 18 male alcoholics were used as subjects, and the measurements of eruptions and erythema produced by intracutaneous reactions to physiological saline, Allergen of 'sake', Sake-gen I and Sake-gen II taken at fixed intervals were processed in detail statistically. As a result, it was found that there were significant differences. Further, it was found that Sake-gen II was not a good test solution.

3. The discriminant analysis theory was applied to the normal male and alcoholics groups, and measurements taken at fixed intervals as in the above case, were used as variables to prepare criteria for both groups, and mention was made as to the possibility of making suggestions as to those who would become alcoholics in the future.

4. Skin tests similar to those above were actually carried out on 361 industrial workers of a certain factory in accordance with the above criteria and the findings were studied. The results indicated that there were as many as 70 whom it was assumed had a possibility of becoming an alcoholic.

Further, prior to the skin tests the 361 workers were subjected to M. P. Manson's Alcohol Addiction Test. Of those who it was assumed there was a possibility of becoming an alcoholic and those who it was difficult to determine as to whether they would or would not become an alcoholic (a total of exactly 100), there were 45 with A-test scores of 20 or more. Those whose various test results and coworkers' evaluations agreed, numbered 13 (43%). This indicates that combining the discriminant method advocated by the author with the A-test, gives very good results.

5. From the various reviews mentioned above, it was possible to point out the roles of the sex and constitutional differences as well as establish objective indices as to the possibility of predicting those who would become alcoholics in the future, and discuss the usefulness in the area of preventive medicine.

6. It may appear as though the problem of alcohol intolerance and skin tests are independent of one another, but actually it cannot be said that these phenomena are totally alien to each another. The reason is that although there are differences in opinion about using skin tests to predict dietary allergies, it is well known that such tests are actually being used routinely in the clinic, and further from the viewpoint of embryology, the skin and the cerebral cortex are both ectodermogenic.

The author plans further to improve the test solutions, to make further study of the data on subjects who underwent repeated tests, make inquiries into histamine test solutions and to establish a discriminant value while giving consideration to the subject's mental aspect, and further develop the discriminant method by conducting studies using both sexes.

Acknowledgement

At the conclusion of this report, the author wishes to express his heartfelt appreciations to his respected teacher Professor Masuho Konuma for his kind guidance, and to Assistant Professor Asada for his kind review of the manuscript.

For the preparation of Sake-gen, appreciations go to Professor Takaaki Aratani, De-

partment of Chemistry, and Assistant Professor Hisanori Okuda and Lecturer Shiro Tsukada of Department of Hygiene, Hiroshima University. He is also indebted to Mr. Takao Shohoji, Department of Statistics, ABCC for statistical processing of the data.

対酒耐性の皮内テストによる体質学的研究

津 久 江 一 郎

広島大学医学部精神神経科学教室 (主任: 小沼十穂教授)

瀬野川病院 (院長: 津久江一郎)

正 法 地 孝 雄

ニューヨーク大学メディカルセンター, 環境医学研究所

(A. B. C. C. 統計部解析員)

(受付: 昭和42年12月12日)

皮内テストに用いた液は, 清酒アレルゲン (鳥居薬品), 酒ゲン, 対照として生塩水の3種類で, 注射后5, 10, 15, 20, 30分値の膨疹, 紅斑の平均直径をもつて計測した。

その結果, 健常男子 (94名) 女子 (81名) 間及び健常男子群と男子アルコール中毒者群 (18名) との間に, 統計学的に著明な差がある事が認められた。

そこで予防医学的な見地より, 将来健常者がアルコール中毒者になる可能性があるかどうかを見る為に判別函数の理論の適用をこゝろみた。これにより, 上述の健常男子群とアルコール中毒者 (男子) 群との間を最も良く区分する為の線型函数を求めた。これを某事業所産業労働者361名に適用すると同時に M. P. Manson の Alcadd. Test をも全員施行した。

その結果, 本テストで26%, Alcadd. Test で50.1%が陽性となり, 両者とも陽性となったものは12.7%であった。

このうち個人的探索により43%が問題酩酊者である事が判明し, 本方法のスクリーニングテストとしての有用性を認めた。

References

- 1) HIMWICH, H. E.: Alcoholism as a medical problem 32, 1956.
- 2) ROBINSON and VOGTLIN: Alcoholism as a medical problem 32, 1956.
- 3) TAKASE, Y.: Skin reactions from the viewpoint of skin physiology. J. Allergy, 12 (1, 2): 4-13, 1963.
- 4) HARGER, R. N.: Absorption of alcohol. Alcoholism, 121 (George N. Thompson), 1956.
- 5) MANSON, M. P.: Quarterly J. of Studies on Alcohol, 9: 175-206, 1948.
- 6) MANSON, M. P.: Apsy chometric determination of alcoholic addiction. Amer. J. Psychiat., 106: 199, 1949.
- 7) URBACH: Alcohol and the skin. Medicine, 11 (3): 165, 1954.
- 8) KISHI, T.: Experimental pathological studies on 'sake'. J. Life Insurance Medicine, 53(3): 25, 1955.
- 9) ANDERSON, T. W.: An introduction to multivariate statistical analysis. John Wiley, 1958.
- 10) RAO, C. R.: Advanced statistical methods in biometric research. John Wiley, 1952.
- 11) KUROYANAGI, T.: Clinical allergy. Igaku Shoin, 1956.
- 12) ARAKAWA, T.: Skin reaction. Modern Medicine, 10(5): 45-53, 1955.
- 13) AOI, A.: Dietary preferences and dermatological functions, particularly Japanese 'sake'. Shikoku Med. J., 16: 855, 1959.
- 14) AOKI, Y., et al.: Psycho-pathological studies on alcohol drunkenness. Psychiatria et Neurologia Japonica 62(4): 791, 1960.
- 15) KUMAGAI, A.: Studies on allergen skin test. J. Allergy, 11: 246 1962.
- 16) KUMAGAI, A.: Basic studies on allergen intracutaneous tests. J. Allergen, 11: 304, 1962.
- 17) ENJOJI, M.: Determination time of intracu-

- taneous reaction, injection site and dose. J. Allergen, 11: 297, 1962.
- 18) ISHIZAKI, T: Intracutaneous reaction. J. Allergen, 12: 14-32, 1963.
- 19) UTSUNOMIYA, S: Physiological variations in eruption absorption time. Dermatology and Urology, 26: 6, 1964.
- 20) TSUKUE, I.: Studies on constitutional tolerance for alcohol through intracutaneous reactions. The Journal of The Hiroshima Medical Association, 18(10), 1965.
- 21) TSUKUE, I.: Studies on constitution of schizophrenics for alcohol. The Journal of The Hiroshima Medical Association, 18(10), 1965.
- 22) TSUKUE, I.: Constitutional studies on alcohol tolerance through skin test II. Skin test on alcoholics. Japan. J. Stud. Alcohol, 1(2): 90~99, 1966.