A STATISTICAL STUDY OF THE EFFECT OF ISONICOTINIC ACID HYDRAZIDE (INH) ON THE ACETYLATION OF SULFANILAMIDE IN RABBITS

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Introduction

The effect of acute and chronic administration of INH on the acetylation of sulfanilamide by rabbits is being studied in the present paper. The rabbit is one of the commonly used laboratory animal for biological studies. For studies requiring withdrawal of blood samples, it is distinctly a more convenient animal as campared to others including mice, rats and guinea-pigs. Being smaller than dogs or monkeys there is economy in their use. It is known that the rabbit can acetylate sulfanilamide to a very high degree. This is in marked contrast to what generally happens in dog.

To study the effect of acute and chronic administration of INH on the acetylation of sulfanilamide by rabbits fifteen (15) adult rabbits (age above six months) were selected randomly for the experiment. Each rabbit was given sulfanilamide in a dose of 50 mg./kg, orally. Blood samples were draw 1 hour and 4 hour after the administration of sulfanilamide.

In both the blood samples free (non-acetylated) and total (free acetylated) sulfanilamide levels were determined by Brattan and Marshall [1] method for estimation of sulfanilamide in blood. The degree of acetylation was calculated from the total and free sulfanilamide level.

2. The Experiment

On these 15 rabbits three estimations were carried out (at an interval of 7 days between two successive estimations) with the dose of sulfanilamide (50 mg./kg. orally).

It was noted that there was marked time to time differences among percentage acetylation values for the same rabbit.

Table 1 shows readings (both at 1 hour and 4 hours) of percent acetylation values of 15 rabbits for three estimations, in 15 rabbits.

TABLE I

Precent Acetylation (1 hour and 4 hours) in 15 rabbits

Time	*One hour*			*Four hours*		
Rabbit No.	*Ist estimation*	*2nd estimation*	*3rd estimation*	*Ist estimation*	*2nd estimation*	*3rd estimation*
1.	10.00	43.03	44.07	30.80	71.40	62.60
2.	33.30	25.00	30.61	35.70	61.50	64.50
3.	17.80	27.27	23.30	51.90	76.90	42.80
4.	6.67	65.70	32.10	32.70	73.30	40.00
5.	3.39	7.70	37.50	47.70	29.70	47.10
6.	64.40	56.50	39.40	100.00	63.30	66.50
7.	70.90	52.00	52.70	100.00	71.40	71.10
8.	38.80	29.42	28.901	00.00	52.70	79.10
9.	50.00	40.65	29.80	97.00	68.50	39.60
10.	50.00	26.70	44.40	92.00	61.35	66.50
11.	73.50	39.75	47.40	100.00	62.60	69.40
12.	79.50	29.30	26.20	100.00	69.00	46.00
13.	66.80	41.00	45.20	100.00	67.00	64.00
14.	64.80	37.00	30.20	79.50	38.00	42.60
15.	74.90	52.40	45.20	100.00	80.00	76.00

Consider the set of values for the first estimation at one hour, namely 10.00, 33.30 and so on. These are 15 values in all. As has already been stated the object of the experiment was to determine the value of percentage of acetylation in rabbits.

It is easy to note that rabbits would depending on their physical compositions to react to the dose. Specifically, reactions of rabbits would depend on factors like, place, temperature, climate and food taken etc. However, in the present experiments the rabbits were kept in controlled conditions so that the effects due to the above mentioned factors might be as negligible as possible.

It is important that for a valid statistical analysis, the rabbits experimented on, should be as representative of the general stock as possible and devoid of any defects or abnormalities. Otherwise the results of the experiment would be true for the rabbits experimented on and not hold good in general. For percentage acetylation at one hour, there are three estimations which were carried out at an interval of seven days. It is of interest to compare readings of estimation generated at different times. If the injection of material into a rabbit's body changes its metabolism then at subsequent estimation

stages the readings would be either increased or decreased. In order to test whether there exists such a change in rabbits, the R.B.D. was applied to the data.

3. APPLICATION OF RANDOMISED BLOCK DESIGN

We are having three estimations on 15 rabbits. If a reading x_{ij} denotes the observation on the i^{th} rabbit at the time of j^{th} estimation $i = 1, 2, \ldots, 15, j = 1, 2, 3$, then x_{ij} denotes the effect corresponding to i^{th} rabbit at the j^{th} estimation. In general x_{ij} could be expressed as

$$x_{ij} = u + \beta_i + T_j + \epsilon_{ij}$$

where u denotes the general mean, β_i stands for the effect due to the i^{th} rabbit, T_j is the effect at the j^{th} estimation. Further, ϵ_{ij} is a random error component with mean zero and $E(\epsilon_{ij}^2) = \sigma^2$. Here we assume that E_{ij} are normally and independently distributed with mean zero and variance σ^2. With this assumption the following hypotheses are tested.

1. H_1 : Rabbits do not differ among themselves.
2. H_2 : Three estimations do not differ among themselves.

The above two hypotheses were tested for control data of Table No. 1 for one hour and four hour separately. The results are as follows:

3. (*a*) *Analysis of Variance Table* (*One hour*) *Control data*

Source	d. f.	S.S	M.S.S	F
Rabbits	14	6843.05	488.79	1.83 (N.S.)
Estimation	2	877.10	438.55	1.64 (N.E.)
Error	28	7468.82	266.74	
Total	44	15188.98		

3. (*b*) *Analysis of Variance Table* (*Four hour*) *Control data*

Source	d. f.	S.S.	M.S.S.	F.
Rabbits	14	7739.23	552.80	1.67 (N.S.)
Estimation	2	3059.68	1529.84	4.62*
Error	28	9236.95	329.891	
Total	44	20035.86		

(*Significant at 5 percent level of significance)

For one hour data differences among three estimations were found to be non-significantat 5 percent level of significance.

For four hour data the three estimations showed significant differences among themselves. Subsequently the following three hypotheses were tested :

(*a*) H_o : Mean of 1st Estimation = Mean of 2nd Estimation

$$t_{error\ d.f.} = \frac{|\bar{X}_i - \bar{X}_j|}{\text{error } m.s.s. \times \left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$$

$$t_{28} = \frac{14.71}{329.89 \times \frac{2}{15}} = 2.21^*$$

From the statistical Table,

$$t_{28,\ 0{\cdot}05} = 2.048$$

The above hypothesis is rejected at 5 percent level of significance.

(*b*) Mean of 1st estimation = Mean of 3rd estimation. By the application of *t* test, it is noted that the hypothesis is rejected at 5 percent level of significance.

(*c*) Mean of 2nd estimation = Mean of 3rd estimation. The above hypothesis is accepted at 5 percent level of significance.

4. ACUTE AND CHRONIC ADMINISTRATION

The same batch of 15 rabbits was administered acute dose (50 mg/kg) only once (both at 1 hour and 4 hours) and chronic dose (10 mg/kg) (both at 1 hour and 4 hours). The readings after acute and chronic administration both at 1 hour and 4 hours are given respectively in table numbers 2 and 3. In order to study the effect of

TABLE 2
Readings for Acute administration

Reading No.	*Acute (1 hour)*	*Acute (4 hours)*
1	33.25	63.40
2	28.60	66·60
3	15.20	71.40
4	23.50	66.20
5	37.40	70.00
6	29.30	79.10
7	38.80	100 00
8	34.10	47.40
9	36.30	66.90
10	43.60	55.60
11	25.90	53.90
12	20.16	66.50
13	33.40	59.20
14	40.70	75.60
15	64.80	77.70

acute and chronic administration, the readings given in table number 1 were considered.

TABLE 3

Readings for Chronic administration

Reading No.	*Chronic (1 hour)*	*Chronic (4 hours)*
1	69.40	100.00
2	54.80	81.20
3	64.40	84.30
4	57.50	78.50
5	59.50	74.00
6	31.70	76.90
7	62.50	81.80
8	died	died
9	44.20	77.50
10	45.50	81.20
11	66.50	100.00
12	33.90	70.90
13	52.80	80.09
14	36.80	57.20
15	36.70	83.90

IV (*a*). Since it was observed that for 1 hour data the three estimations do not differ significantly among themselves, the data consisting of the three estimations for control for one hour data of Table No. 1 and the estimation due to Acute treatment for one hour of Table No. 2 was analysed again using a Randomised Block Design. The following hypothesis was treated :

H_0 : The four estimations do not differ among themselves.

The results are as follows :

A.V.T. (One hour, Acute)

Source	*d. f.*	*S.S.*	*M.S.S.*	*F.*
Rabbits	14	5914.48	422.46	1.72 (N.S.)
Estimation	3	1444.00	481.33	1.96 (N.S.)
Error	42	10294.35	245.10	
Total	59	17652.83		

Subsequently from the above analysis, it is noted that the effect due to acute dose is the same as that due to control. Same analysis was carried out with Chronic treatment. In the case of Chronic administration, reading on 8th rabbit was not available. Hence observations on 14 rabbits were considered.

The results are as follows :

A.V.T. (One hour Chronic)

Source	*d. f.*	*S.S.*	*M.S.S.*	*F.*
Rabbits	13	4150.65	319.28	1.02 (N.S.)
Estimation	3	1863.20	621.06	1.98 (N.S.)
Error	39	12257.58	314.29	
Total	55	18271.4303		

Here also there does not exist any significant effect at the 5 percent level, between the administration of chronic dose and the control.

For four hour data, using the same technique, Acute and Chronic treatments were tested with the 2nd and 3rd estimations of control data for four hour of Table No. 1. The results are as follows :

A.V.T. (Acute)

Source	*d. f.*	*S.S.*	*M.S.S.*	*F.*
Rabbits	14	2892.00	206.57	1.23 (N.S.)
Estimation	2	669.47	334.73	2.01 (N.S.)
Error	28	466.69	166.70	
Total	44	8229.16		

N.S. Not significant at 5 percent level of significance.

For 4 hour readings it is noted that there does not exist and significant difference between the acute and the control readings.

A.V.T. (Chronic)

Source	*d. f.*	*S.S.*	*M.S.S.*	*F.*
Rabbits	13	3804.10	292 62	3.06*
Estimation	2	4086.07	2043.03	21.42*
Error	26	2479.57	95.36	
Total	41	10369.74		

*Significant at 5 percent level of significance. Here also at 5 percent level of significance, the effect due to chronic as compared to the control is significant.

5. SIGN TEST

5(a). One hour data

H_{01} : 1st and 2nd estimations are from the same population in one hour data.

N = Total No. of pairs, d_i = difference for the i^{th} pair.

K = Total number of fewer signs obtained for the differences d_i, $i = 1,2,...,15$.

$= 4$

Prob. $[k \leqslant 4] = 0.059 > 0.05$

Hence the hypothesis is not rejected at 5 percent level of significance.

5(*b*).

H_{02} : 2nd and 3rd estimations are from the same populations.

$N = 15$, $K = 7$

Prob. $[k \leqslant 7] = 0.500 > 0.05$

Hence the hypothesis H_{02} is not rejected.

Difference between 2nd and 3rd estimations is considered to be non-significant at 5 percent level of significance.

5 (*c*).

H_{03}: 1st and 3rd estimations are from the same populations.

$N = 15$, $K = 4$

Prob. $[k \leqslant 4] = 0.059 > 0.05$

H_{03} is not rejected.

Hence difference between 1st and 3rd estimations is considered to be non-significant, at 5 per cent level of significance.

Four hour control data

5(*d*). H_{01} : 1st and 2nd estimations are from the same populations.

$N = 15$, $K = 4$

Prob. $[k \leqslant 4] = 0\ 059 > 0.05$

H_{01} is accepted at 5 percent level of significance.

Hence there does not exist significant difference between 1st and 2nd estimation at 5 percent level of significance.

5(*e*). H_{02} : 2nd and 3rd estimations are from the same populations.

$N = 15$, $K = 7$

Prob. $[k \leqslant 7] = 0.500 > 0.05$

H_{0_2} is accepted at 5 percent level of significance.

There does not exist significant difference between 2nd and 3rd estimation at 5 percent level of significance.

5(f) H_{03} : 1st and 3rd estimations are from the same populations.

$N=15$,

$K=3$

Prob. $[k\leqslant 3]=0.018<0.05$

H_{03} is rejected at 5 percent level of significance.

There exists significant difference between 1st and 3rd estimation at 5 percent level of significance. Hence for further comparison with Acute and Chronic treatments, group mean of 1st, 2nd and 3rd estimation of one hour data and mean of 2nd and 3rd estimation of 4 hour data is considered.

5(g). H_{11}: There is no significant difference between group means of 1st, 2nd and 3rd estimations for one hour data given in Table No. 1 and readings for Acute treatment for one hour data of Table No. 2. By the application of sign test, we get

$N=15$, $K=5$

Prob. $[k\leqslant 5]=0.15>0.05$

At 5 percent level of significance, the hypothesis H_{11} is accepted.

5(h). H_{12}: There is no significant difference between group means of 1st and 2nd and 3rd estimations for one hour data given in Table No. 1 and readings for chronic treatment for one hour data of Table No. 2. By the application of sign test, we get

$N=14$, $K=4$

Prob. $[k\leqslant 4]=0.090>0.05$

At 5 percent level of significance the hypothesis H_{12} is accepted.

5(i). H_{21}: There is no significant difference between group means of 2nd and 3rd estimations for four hour data of Table No. 1 and readings for Acute treatment for four hour data of Table No. 2. By the application of sign test, we get,

$N=15$, $K=6$

Prob. $[k\leqslant 6]=0.304>0.05$

At 5 percent level of significance, the hypothesis H_{21} is accepted.

5(j). H_{22}: There is no significant difference between group means of 2nd and 3rd estimations for four hour data of Table No. 1

and readings for Chronic treatment for four hour data of Table No. 2. By the application of sign test, we get,

$$N=14,\ K=0$$

Prob. $[k \leqslant o] < 0.05$

At 5 percent level of significance the hypothesis H_{22} is rejected.

These results are presented in the following table. :

TABLE 4

Presentation of Result

Comparison	*Conclusion*
ONE HOUR	
1. Group mean of three estimations with acute treatment.	The two populations are the same.
2. Group mean of three estimations with chronic treatment.	The two populations are the same.
FOUR HOUR	
1. Group mean of 2nd and 3rd Eastimation with acute treatment.	The two population are the same.
2. Group mean of 2nd and 3rd estimations with chronic treatment.	The two populations are not same.

6. SUMMARY

The effect of acute and chronic administration of INH on the acetylation of sulfanilamide by rabbits is being studied in the present paper. The experiment was carried on 15 rabbits by taking blood samples at 1 hour and 4 hour after the administration of sulfanilamide. For each rabbit the degree of acetylation was calculated.

On 15 available rabbits three estimations were carried out at an interval of 7 days before applying the treatment. Since the control readings showed differences among themsleves first it was tested, whether the control readings differ among themselves. The analysis was done by using a R.B.D. and the sign test. From the applications of R.B.D. for 1 hour data, it was observed that the three estimations did not differ among themselves. Hence all the three estimations were considered while comparing the Acute treatment. Again applying a R.B.D. it was observed that iffect of Acute and Chronic administration was the same as due to the control.

For 4 hour data when a R.B.D. was applied only 2nd and 3rd estimations did not show any significant differences among themselves. Hence for comparison with Acute and Chronic treatment 2nd and 3nd estimations of control readings were considered. It was observed that effect Chronic dose only was significant at 5 percent level of significane.

Analysis was carried out by the application of sign test. For one hour data it was observed that three estimations did not show significant differences among themselves. For further comparison group means of these three estimations were considered. In this case effects of both Chronic and Acute treatments were the same as due to the control.

For 4 hour data it was observed that there did not exist significant difference between 2nd and 3rd estimations. Hence Acute and Chronic treatments were compared with group means of 2nd and 3rd estimations only. It was noted that only Chronic treatment showed significent effect when compared with control readings.

7. Acknowledgement

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Reference

[1] Marshall : *Journal of Biological Chemistry*. 122 : 263 (1937).