CHAPTER V

R E S U L T S

CHAPTER V

RESULTS

Results have been analysed by Student's* 't' test. In experiment 1, comparison has been made between (i) normal group and other groups (ii) diet group and drug treated groups and (iii) tolbutamide group and other drug treated groups. In experiment 2 and 3, results have been compared independently in each set. Control group has been compared with insulin and tolbutamide group. Comparison of insulin group with tolbutamide group has also been done in each set.

Experiment 1 :

Mean age, weight, income, haemoglobin level, blood pressure, number of smokers and incidence of cardiovascular disease in each group are shown in Table 1.

Mean age in all groups varied from 41.8 to 47.6 years and mean weight from 128.3 lbs. to 147.1 lbs. Mean income per month varied from Rs.229 to Rs.383. Mean haemoglobin level and blood pressure did not vary much and were found to be within normal limits.

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*Results were checked on a TDC-12 Computer at Gujarat State Fertilizers Co.Ltd., Baroda, India. 'T' tables were referred from 'Statistical Methods' by George W. Snebecar, published by Allied Pacific Pvt. Ltd. (Indian edition, 1961).

TABLE 1

Table showing mean, age, weight, income, haemoglobin level, blood pressure, number of smokers and percentage of persons showing history of cardiovascular disease in each group.

Sr. No.	Group	Age yrs.	Weight lbs.	Income Rs.	Mean Hb gms%	Mean B.P. mm of Hg	% of smokers	% of persons with history of C.V.D.
I	Normal (20)*	46.7	135.5	271	13.5	120/80	-	-
II	Freshly detected (21)	41.8	133.9	289	12.4	130/80	19.04% (4 persons out of 21)	19.05% (4 out of 21)
III	Diet (21)	45.6	128.3	229	12.1	138/80	28.57% (6 out of 21)	9.52% (2 out of 21)
IV	Tolbutamide (37)	47.6	133.5	272	12.6	120/80	35.13% (3 out of 37)	16.21% (6 out of 37)
V	Chlorpropamide (23)	47.4	147.1	313	12.1	130/90	17.39% (4 out of 23)	17.39% (4 out of 23)
VI	Phenformin (22)	46.8	142.3	383	13.1	120/80	27.27% (6 out of 22)	13.63% (3 out of 22)
VII	Combination of chlorpropamide & phenformin (20)	47.3	139.9	367	12.7	140/80	15% (3 out of 20)	15% (3 out of 20)

* Figures in the parenthesis indicate number of observations.

Patients taking chlorpropamide, combination of chlorpropamide and phenformin and freshly detected diabetic patients had lesser proportion of smokers than diet controlled, tolbutamide and phenformin drug treated. Percentage of patients who developed heart disease after drug treatment seemed to be lesser in diet controlled diabetics as compared to groups taking tolbutamide, chlorpropamide, phenformin and combination of phenformin and chlorpropamide.

Table 2 shows mean fasting blood sugar level, serum cholesterol, serum triglycerides and serum phospholipid levels. Mean fasting blood sugar of all the groups ranged from 86.5 to 168.4 mg/100 ml of blood. Except for the freshly diagnosed group, blood sugar level was within normal limits.

Serum phospholipid levels were significantly higher in case of phenformin group and group taking combination of phenformin and chlorpropamide ($P < 0.05$ and $P < 0.005$ respectively) than normal group. As shown in Table 3, other comparisons did not show significant difference in serum phospholipid levels.

TABLE 2

Table showing mean of fasting blood sugar, serum phospholipids, triglycerides and cholesterol level in each group.

Sr. No.		Fasting blood sugar mg/100 ml	Phospholipids mg/100 ml	Triglycerides mg/100 ml	Cholesterol mg/100 ml
I	Normal	86.5 ± 14.09 (20)	166 ± 21.64 (20)	112 ± 34.40(20)	178 ± 50.00 (20)
II	Freshly diagnosed	168.4 ± 35.83 (21)	181 ± 48.48 (21)	153 ± 16.56 (21)	247 ± 53.39 (21)
III	Diet controlled	92 ± 18.88 (21)	176 ± 38.42 (21)	96 ± 39.29(21)	176 ± 32.82 (21)
IV	Tolbutamide	102.1 ± 22.13 (37)	184 ± 38.32 (36)	177 ± 100.98(37)	214 ± 34.60 (37)
V	Chlorpropamide	101 ± 28.73 (23)	174 ± 32.57 (22)	160 ± 69.02 (23)	197 ± 44.87 (23)
VI	Phenformin	96.8 ± 16.59 (22)	187 ± 38.43 (22)	123 ± 76.27 (22)	181 ± 22.69 (22)
VII	Combination of chlorpropamide and phenformin	99.4 ± 23.37 (21)	196 ± 32.83 (21)	153 ± 55.57 (21)	195 ± 34.93 (21)

Values are mean ± standard deviation. Values in the parenthesis indicate total number of samples.

TABLE 3

Table showing 't' and 'p' values for comparisons between various groups for the fasting blood sugar, phospholipids, triglycerides and cholesterol.

Sr. No.	Comparisons of groups	Fasting blood sugar 't'	Fasting blood sugar 'p'	Phospholipids 't'	Phospholipids 'p'	Triglycerides 't'	Triglycerides 'p'	Cholesterol 't'	Cholesterol 'p'
I	Normal								
II	Freshly detected VS I	-9.55	P < 0.001	-1.27	-	-2.18	P < 0.05	-4.26	P < 0.001
III	Diet controlled VSI	-1.06	-	1.00	-	1.55	-	0.18	-
IV	Tolbutamide								
	i) VS I	-2.87	P < 0.01	-1.92	-	-2.78	P < 0.01	-3.17	P < 0.005
	ii) VS III	-1.77	-	-0.38	-	-3.55	P < 0.001	-3.86	P < 0.001
V	Chlorpropamide								
	i) VS I	-2.01	-	-0.87	-	-2.79	P < 0.01	-1.85	-
	ii) VS III	-1.21	-	-0.20	-	-3.85	P < 0.001	-1.82	-
	iii) VS IV	0.18	-	1.04	-	0.72	-	1.56	-
VI	Phenformin								
	i) VS I	-2.09	-	-2.18	P < 0.05	-0.60	-	-0.27	-
	ii) VS III	-0.63	-	-0.98	-	-1.51	-	-0.63	-
	iii) VS IV	0.99	-	-0.33	- -	2.14	P < 0.05	3.87	P < 0.001
VII	Phenformin + Chlorpropamide								
	i) VS I	-2.02	-	-3.40	P < 0.005	-2.85	P < 0.01	-1.31	-
	ii) VS II	-1.13	-	-1.79	-	-4.07	P < 0.001	-1.89	-
	iii) VS IV	0.45	-	1.18	-	0.98	-	1.91	-

't' denotes calculated values of 't'. 'p' value denotes probability. P values are mentioned wherever the difference is significant.

Serum triglyceride levels differed markedly in some of the groups (fig. 2). When compared with normal group, freshly detected group showed significantly higher level of serum triglycerides ($P < 0.05$). Similarly, tolbutamide group showed highly significant difference in triglyceride levels when compared with the normal group ($P < 0.01$). Chlorpropamide group and group taking combination of phenformin and chlorpropamide also showed statistically significant difference when compared with normal group ($P < 0.01$). Diet controlled group and phenformin group did not show any significant difference in serum triglycerides when compared with normal group.

When compared with diet controlled group, tolbutamide and chlorpropamide group showed significantly higher levels of serum triglycerides than the diet controlled group ($P < 0.001$ and $P < 0.001$ respectively). Group taking combination of chlorpropamide and phenformin also showed higher levels of serum triglycerides than the diet controlled group ($P < 0.001$). However, phenformin group did not show significant difference in serum triglyceride levels as compared to diet controlled group.

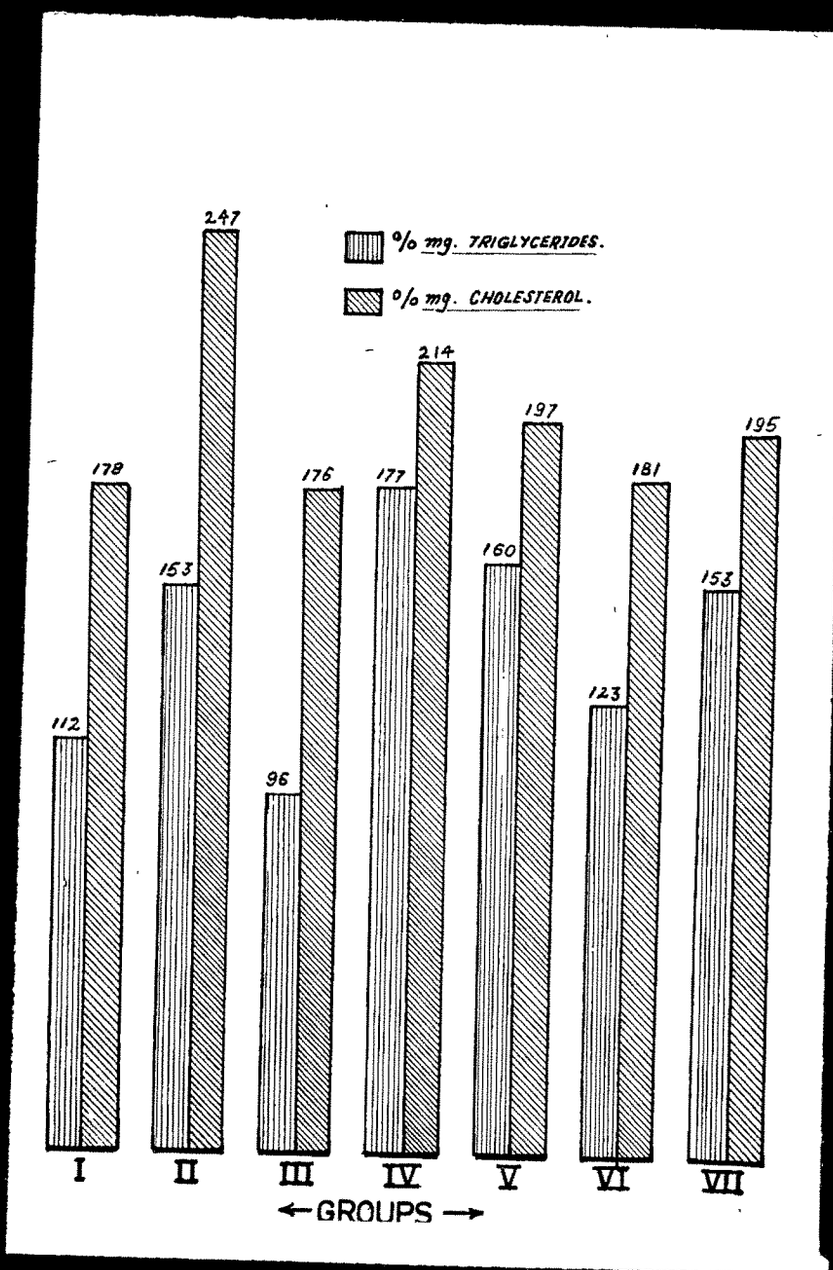

Fig.2 : Serum Triglyceride and Cholesterol Level in Various Groups

Groups : 1. Normal 2. Freshly detected. 3. Diet Controlled.
4. Tolbutamide. 5. Chlorpropamide. 6. Phenformin.
7. Combination of Chlorpropamide and Phenformin.

When compared with tolbutamide group, phenformin group showed significantly lower levels of triglycerides ($P < 0.05$) whereas combination group and chlorpropamide group did not show any difference statistically.

As regards to the serum cholesterol level (fig.2), freshly detected patients and tolbutamide treated patients showed significantly higher cholesterol as compared to normal group ($P < 0.001$ and $P < 0.005$ respectively). Other groups did not show significant difference when compared with the normal group.

When compared with diet controlled group, tolbutamide group showed significantly higher serum cholesterol levels ($P < 0.001$) whereas patients taking chlorpropamide, phenformin and combination of the two did not show any statistically significant difference when compared with diet controlled group.

As shown in table 2 and 3, tolbutamide group showed significantly higher cholesterol levels as compared to phenformin group ($P < 0.001$). Chlorpropamide group did not show significant difference when

compared with tolbutamide group. Combination group (group VII) showed no significant difference when compared with tolbutamide group.

Figures 3,4 and 5 show lipoprotein patterns on cellogel strips. Table 4 indicates lipoprotein pattern as well as percentage of type II and IV patients in each group. It may be noted that type II and type IV were found to be more common in diabetes as a whole. Diet group had lesser number of abnormal cases as compared to sulfonylurea groups. None of the abnormal patterns were found in phenformin group. However, freshly detected tolbutamide group and group receiving combination of chlorpropamide and phenformin showed higher incidence of type II abnormality. Combination group also showed 25 percent cases of type IV.

As shown in Table 5, lipoproteins (expressed as percentage of peak area) were significantly higher as compared to normal group except that phenformin group did not show significant differences in alpha and pre-beta lipoproteins levels as compared to normal group.

LIPOPROTEIN PATTERNS ON CELLOGEL STRIPS

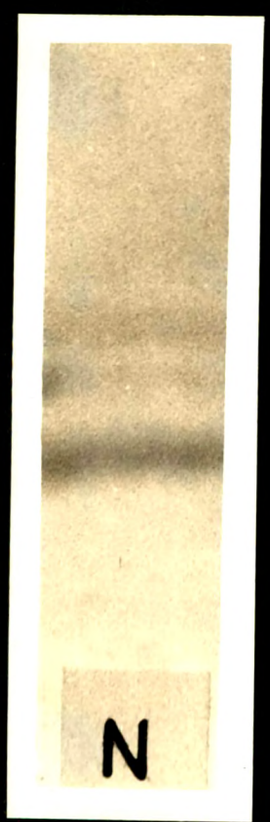

Fig.3 Normal

Fig.4 Type II

Fig.5 Type IV

TABLE 4

Table showing percentage of patients with type II and type IV (according to Fredrickson and Lees (1965) Classification) and mean alpha, pre-beta and beta-lipoprotein percentage.

Sr. No.	Group	Percentage of type II	Percentage of type IV	* Lipoproteins (percentage of peak area)		
				α	pre-β	β
I.	Normal	-	-	10.28 ± 3.08 (20)	16.06 ± 3.20 (20)	63.17 ± 5.98 (20)
II.	Freshly diagnosed	14.286 (3 out of 21)	-	13.83 ± 5.46 (20)	35.97 ± 7.77 (20)	50.20 ± 7.65 (20)
III.	Diet controlled	-	4.761 (1 out of 21)	17.49 ± 3.56 (20)	27.73 ± 5.13 (20)	54.79 ± 6.05 (20)
IV.	Tolbutamide	13.513 (5 out of 37)	8.108 (3 out of 37)	15.19 ± 1.97 (20)	35.60 ± 8.28 (20)	49.22 ± 8.21 (20)
V.	Chlorpropamide	4.391 (1 out of 23)	-	23.77 ± 5.24 (20)	26.00 ± 4.02 (20)	48.81 ± 4.85 (20)
VI.	Phenformin	-	-	11.64 ± 1.99 (20)	18.23 ± 4.02 (20)	70.14 ± 4.63 (20)
VII.	Combination of Chlorpropamide and Phenformin	10 (2 out of 20)	25 (5 out of 20)	10.71 ± 2.52 (20)	18.50 ± 3.21 (20)	70.80 ± 4.92 (20)

* Values are means of 20 samples

Note : Values of lipoproteins are mean ± S.D.
Figures in parenthesis indicate the number of observations.

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Table showing 't' and 'p' values for comparison between various groups for alpha, beta and pre-beta lipoproteins.

Comparison of groups	Alpha-Lipoproteins		Pre-beta Lipoproteins		Beta-Lipoproteins	
	't'	'p'	't'	'p'	't'	'p'
I Vs II	-2.53	P<0.025	-10.58	P<0.001	9.60	P<0.001
I Vs III	-6.84	P<0.001	-8.63	P<0.001	6.98	P<0.001
I Vs IV	-5.75	P<0.001	-9.84	P<0.001	6.77	P<0.001
I Vs V	-9.91	P<0.001	-4.59	P<0.001	13.17	P<0.001
I Vs VI	-1.65	Not significant	-1.88	Not significant	-6.51	P<0.001
I Vs VII	-0.76	Not significant	-3.81	P<0.001	-7.00	P<0.001

't' denotes calculated values of 't'. 'p' denotes probability.
P values are given only where the difference is significant.

Experiment 2 :

All results were statistically analysed using student's 't' test. It may be noted that because of high biological variation and small number of animals in each group with comparatively short periods of treatment, it was difficult to produce pronounced effect and observe statistically significant difference for each parameter. However, there was distant difference in tissue lipid patterns in response to insulin/tolbutamide treatment. These were remarkably different from control. Attempt has been made to compare treatment groups with control. The differences between the treatment groups have also been studied.

Table 6 shows initial body weights, final body weights and mean weight gain of the rabbits. It can be well observed that there was no noticeable difference in the net gain in weight of the animals subjected to either of the treatment (insulin/tolbutamide).

Table 7 shows mean blood sugar, serum cholesterol and phospholipid levels of rabbits at 0 week and 13th week (final level). It can be noted that blood sugar concentration did not show significant

TABLE 6

Initial body weights, final body weights and mean weight gain of rabbit treated with insulin/tolbutamide with and without cholesterol feeding.

No.	Treatment	Initial weight kg	Final weight kg	Mean weight gain kg
Set A				
I	Control	1.60	2.40	0.80
II	Insulin	1.60	2.36	0.74
III	Tolbutamide	1.59	2.56	0.80
Set B				
IV	Cholesterol fed control	1.50	2.40	0.90
V	Insulin + Cholesterol	1.70	2.50	0.80
VI	Tolbutamide + Cholesterol	1.63	2.30	0.70

TABLE 7

Mean blood sugar, serum cholesterol and phospholipid levels at various intervals in insulin/tolbutamide treated rabbits.

Group	0 Week			13th Week		
	Sugar mg/100 ml	Cholesterol mg/100 ml	Phospho-lipids mg/100 ml	Sugar mg/100 ml	Cholesterol mg/100 ml	Phospho-lipids mg/100 ml
Set 'A'						
I Control	65.3 ± 17.46 (6)	60.3 ± 19.9 (6)	65 ± 24.5 (6)	75.3 ± 9.4 (6)	41 ± 7.9 (6)	75 ± 21.5 (6)
II Insulin	74 ± 13 (6)	50.6 ± 11.3 (6)	82 ± 26.7 (6)	60 ± 13.8 (6)	36 ± 3.0 (6)	133 ± 29 (6)
III Tolbutamide	45.6 ± 10.90 (6)	43 ± 15.40 (6)	48.3 ± 15.00 (6)	52 ± 14.00 (6)	70 ± 6.2 (6)	212 ± 18 (6)
Set 'B'						
IV Control + Cholesterol	68 ± 17.20 (6)	41.6 ± 3.2 (6)	73.3 ± 13.3 (6)	53.3 ± 4.80 (6)	1046 ± 126 (6)	145 ± 43 (6)
V Insulin + Cholesterol	57.3 ± 13.55 (6)	50.7 ± 11.7 (6)	57 ± 24.8 (6)	62.8 ± 17.39 (6)	1265 ± 69 (6)	312 ± 39.9 (6)
VI Tolbutamide + Cholesterol	57.8 ± 14.38 (6)	51.8 ± 5.4 (6)	123 ± 21.7 (6)	61.3 ± 14.94 (6)	1001 ± 133 (6)	210 ± 49.4 (6)

Values are means ± S.D.. Figures in the parenthesis indicate number of observations.

change in either insulin or tolbutamide group in set 'A' (without cholesterol) and set 'B' (with cholesterol) throughout the experimental period.

Final serum cholesterol levels did differ from the initial levels between the various groups in set 'A'. Group II (insulin treated) showed significant decrease at the end of experiments ($P < 0.025$) whereas group III (tolbutamide treated) showed significant increase in serum cholesterol ($P < 0.005$). In set 'B', all the groups showed significantly higher cholesterol levels at the end of experiment as compared to their corresponding initial levels in all cases ($P < 0.001$).

As shown in tables 7 and 8, serum phospholipids increased to a very significant extent at the end of experimental period in all the groups except control group. 'P' values are given in table 8. However, it may be noted that the difference between initial and final levels of serum phospholipids was more pronounced in case of group III and group V ($P < 0.001$).

Table 9 shows mean blood sugar, serum proteins, serum cholesterol, phospholipids and

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Table showing 't' and 'p' value for comparison between various groups of blood sugar, serum cholesterol and serum phospholipids.

Initial Vs final levels	Blood Sugar		Serum Cholesterol		Serum Phospholipids	
	't'	'p'	't'	'p'	't'	'p'
Set A						
Group I (Control)	-1.234	-	2.205	-	-0.750	-
Group II (Insulin)	1.806	-	3.050	P<0.025	-3.165	P<0.025
Group III (Tolbutamide)	-0.871	-	-3.975	P<0.005	-17.047	P<0.001
Set B						
Group IV (Control + Cholesterol)	2.009	-	-19.550	P<0.001	-3.897	P<0.005
Group V (Insulin + Cholesterol)	-0.61	-	-42.720	P<0.001	-13.274	P<0.001
Group VI (Tolbutamide + Cholesterol)	-0.41	-	-17.508	P<0.001	-3.947	P<0.005

't' denotes calculated values of 't'. 'p' value denotes probability. P values are given only when the difference is significant.

TABLE 9

Mean blood sugar, serum proteins, serum cholesterol, phospholipids and triglycerides of insulin/tolbutamide treated rabbits at the end of experimental period.

Group	Blood Sugar mg/100 ml	Serum Proteins gm/100 ml	Serum Cholesterol mg/100 ml	Serum Phospholipids mg/100 ml	Serum triglycerides mg/100 ml
Set 'A'					
I Control	75.3 ± 9.4 (6)	7.76 ± 0.87 (6)	41 ± 7.9 (6)	75 ± 21.5 (6)	83 ± 24.25 (6)
II Insulin	60 ± 13.8 (6)	6.8 ± 1.40 (6)	36 ± 3.0 (6)	133 ± 29 (6)	79 ± 12.0 (6)
III Tolbutamide	52 ± 14 (6)	6.78 ± 0.70 (6)	70 ± 6.2 (6)	212 ± 18 (6)	98 ± 15.0 (6)
Set 'B'					
IV Cholesterol	53.3 ± 4.8 (6)	8.7 ± 1.77 (6)	1046 ± 126 (6)	145 ± 43 (6)	531 ± 53 (6)
V Insulin + Cholesterol	62.8 ± 17.39 (6)	8.08 ± 1.99 (6)	1265 ± 69 (6)	312 ± 39.9 (6)	585 ± 93.5 (6)
VI Tolbutamide + Cholesterol	61.3 ± 14.94 (6)	8.98 ± 1.21 (6)	1001 ± 133 (6)	210 ± 49.4 (6)	550 ± 90.2 (6)

Values are means ± S.D.. Figures in the parenthesis indicate number of observations.

triglyceride levels at the end of experimental period. Group II and group III showed significantly lower levels of blood sugar than group I ($P < 0.05$ and $P < 0.01$) respectively (Table 10).

No significant difference was found in final blood sugar levels of the other groups. Serum protein levels also showed no significant difference between all the groups. Serum cholesterol levels were significantly higher in group III i.e. tolbutamide group ($P < 0.001$). Group III also showed significantly higher level of cholesterol than group II ($P < 0.001$).

In set 'B', group V showed significantly higher cholesterol levels than group IV ($P < 0.005$) and group VI ($P < 0.005$).

As regards serum phospholipid levels, group II (insulin group) had significantly higher phospholipid levels than the control group ($P < 0.005$). Group III had significantly higher phospholipid levels than the control group ($P < 0.001$). Group III also showed significantly higher level of phospholipids when compared with group II ($P < 0.001$). In set 'B', group V had significantly higher phospholipid levels

TABLE 10

Table showing 't' and 'p' values for comparison between various groups for blood sugar, serum proteins, serum cholesterol, serum phospholipids and serum triglycerides

Group	Blood Sugar		Serum Proteins		Serum Cholesterol		Serum Phospho-lipids		Serum Trigly-cerides	
	't'	'p'	't'	'p'	't'	'p'	't'	'p'	't'	'p'
Set A										
I VS II	2.246	P < 0.05	1.399	-	1.439	-	-3.924	P < 0.005	-0.33	-
I VS III	3.385	P < 0.01	2.155	-	-7.033	P < 0.001	-11.933	P < 0.001	-1.11	-
II VS III	0.995	-	0.008	-	-12.021	P < 0.001	- 5.651	P < 0.001	-2.209	-
Set B										
IV VS V	-1.30	-	-0.566	-	-3.743	P < 0.005	- 6.964	P < 0.001	-1.122	-
IV VS VI	-1.25	-	-0.322	-	0.602	-	- 2.430	P < 0.05	-0.407	-
V VS VI	0.1602	-	-0.944	-	4.229	P < 0.005	3.931	P < 0.005	-0.603	-

't' denotes calculated values of 't'. 'p' values denotes probability.
P values are given only where the difference is significant.

than group IV (control) ($P < 0.001$). Group VI (cholesterol + tolbutamide) showed significantly higher levels as compared to group IV (control) ($P < 0.05$). Group V (Insulin + cholesterol) showed significantly higher levels of serum phospholipids than group VI ($P < 0.005$). Serum triglyceride levels were not found to differ significantly in any of the groups when compared with control group.

Table 11 shows weight of the liver, total fat, total and free cholesterol, phospholipids and triglycerides. There was no significant difference in liver weights in all the groups (both the sets) when compared to their controls except group II (Table 12). In group II, liver weight was significantly higher than group I ($P < 0.05$).

There was no change in the total lipids of the liver except for group II which showed significantly higher total lipids as compared to group I ($P < 0.025$). Free cholesterol in liver was significantly higher in group III as compared to group I ($P < 0.025$). Similar trend was observed in set 'B' where free cholesterol level of group VI was higher than group IV and V ($P < 0.001$ and $P < 0.025$) respectively.

TABLE 11

Mean liver weights, total fat, free and total cholesterol, phospholipids and triglycerides of insulin/tolbutamide treated rabbits.

Group	Liver weights gm/100 ml	Total lipids gm%	Cholesterol Free mg%	Cholesterol Total mg%	Phospho-lipids mg%	*Triglycerides mg%
Set 'A'						
I Control	45.8 ± 7.2 (6)	4.42 ± 0.28 (6)	135 ± 37.6 (6)	254 ± 52.5 (6)	1239 ± 569 (6)	293
II Insulin	52.7 ± 1.9 (6)	5.33 ± 0.68 (6)	161 ± 30.1 (6)	294 ± 45.9 (6)	912 ± 336 (6)	413
III Tolbutamide	47.6 ± 10.1 (6)	8.11 ± 1.26 (5)	162.6 ± 14.9 (6)	270 ± 105.4 (6)	1194 ± 308 (6)	658
Set 'B'						
IV Cholesterol	72.2 ± 16.1 (6)	11.18 ± 0.96 (6)	340.7 ± 67.3 (6)	2154 ± 815 (6)	1174 ± 254 (6)	723
V Insulin + Cholesterol	76.5 ± 8.0 (6)	10.64 ± 0.80 (6)	366.8 ± 50.92 (5)	2183 ± 455 (6)	1108 ± 715 (6)	745
VI Tolbutamide + Cholesterol	75.4 ± 9.3 (6)	10.97 ± 1.76 (5)	536 ± 11.98 (6)	1553 ± 785 (6)	1458 ± 273 (6)	718

Values are means ± S.D.. Figures in the parenthesis indicate number of observations.

* Since these are derived values, standard deviation is not given.

TABLE 12

Table showing 't' and 'p' values for comparisons between various groups for liver weights, total lipids, free cholesterol, total cholesterol and phospholipids.

Group	Liver weights		Total lipids		Free Cholesterol		Total Cholesterol		Phospho-lipids	
	't'	't'	't'	'p'	't'	'p'	't'	'p'	't'	'p'
Set 'A'										
I VS II	-2.267	P<0.05	-3.004	P < 0.025	-1.634	-	-1.403	-	1.214	-
I VS III	1.201	-	-2.11	-	-2.753	P<0.025	-0.150	-	0.170	
II VS III	-0.456	-	0.858	-	-0.152	-	0.456	-	-1.516	-
Set 'B'										
IV VS V	-0.583	-	1.069	-	-0.555	-	0.073	-	0.211	-
IV VS VI	-0.425	-	0.166	-	-6.993	P < 0.001	1.27	-	-1.865	-
V VS VI	0.209	-	0.271	-	-2.889	P < 0.025	1.99	-	-1.118	-

't' denotes calculated values of 't'. 'p' denotes probability. P values are given only where the difference is significant.

As regards the total cholesterol content, there was no significant difference in the total cholesterol levels of set 'A'. In set 'B' also, no statistically significant difference was found in liver total cholesterol levels of insulin as well as the tolbutamide group when compared with control group (group IV).

Liver phospholipid levels were not found to be significantly different in any of the groups in both the sets of experiments (Table 12).

Table 13 shows lipid composition of aorta. Total lipid levels in aorta in insulin group (group II) are significantly higher than that of control group ($P < 0.05$) whereas tolbutamide group (group III) did not show statistically significant difference when compared with control. In set 'B', no statistically significant difference was found in any of the groups (Table 14).

It may be noted that in case of total cholesterol level of aorta, insulin group (group II) showed statistically significant difference as compared to group I i.e. control group ($P < 0.01$).

TABLE 13

Mean total lipids and total cholesterol levels of aorta in insulin/tolbutamide treated rabbits.

Group	Total lipids gm/100 gm of tissue	Total cholesterol mg/100 gm of tissue
Set A		
I Control	11.16 ± 4.2 (6)	54.0 ± 32.0 (6)
II Insulin	17.18 ± 4.8 (6)	108.0 ± 29.9 (6)
III Tolbutamide	15.4 ± 6.0 (6)	85.0 ± 21.6 (6)
Set B		
IV Cholesterol	10.83 ± 2.53 (6)	296.0 ± 97.6 (6)
V Insulin + Cholesterol	15.0 ± 6.5 (6)	359.0 ± 100.4 (6)
VI Tolbutamide + Cholesterol	13.3 ± 8.6 (6)	256.0 ± 55.0 (6)

Values are means ± S.D. Figures in the parenthesis indicate number of observations.

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Table showing 't' and 'p' values for comparisons between various groups for total lipids and total cholesterol of aorta.

Group	Total lipids		Total cholesterol	
	't'	'p'	't'	'p'
Set 'A'				
I VS II	-2.302	P < 0.05	-3.008	P < 0.01
I VS III	-1.411	-	-1.983	-
II VS III	0.562	-	1.499	-
Set 'B'				
IV VS V	-1.459	-	-1.09	-
IV VS VI	-0.675	-	1.22	-
V VS VI	-0.386	-	2.20	-

't' denotes calculated values of 't'. 'p' denotes probability. P values are given only where the difference is significant.

Tolbutamide group did not show statistically significant difference as compared to group I. In set 'B' no statistically significant difference was found in any of the groups.

Results of histopathological examination :

The control group did not produce any lesions in the aorta or heart nor in liver or kidney (fig.6).

The rabbits treated with insulin alone did not show any evidence of lesions in heart, aorta, kidney and liver except in one rabbit which showed chronic glomerulosclerosis in the kidney.

The group of tolbutamide treated rabbits did not show any significant lesion in the heart or aorta. In two rabbits, there was evidence of fatty infiltration in the liver as well as kidney. The lesions were suggestive of a lipemic state but without affection of blood vessels.

The lesions of only cholesterol treated group showed evidence of fatty infiltration of liver. The kidneys and heart did not show any noticeable lesions. Aorta showed presence of plaques distributed mainly in the arch of the aorta and ascending aorta.

Plaques were not ulcerated and the lesions fell in grade one^{+}.

The group treated with insulin and cholesterol showed fatty infiltration of the liver and myocardium. The aorta had atherosclerotic lesions of ++ severity to ++++ severity. The atheromatous plaques showed evidence of lipid infiltration and hyalinization which means that the plaque was ulcerated and fibre was deposited and later hyalinized. Kidney showed no changes (fig. 7,8,9).

Group receiving tolbutamide and cholesterol showed extensive fatty infiltration, some necrosis and periportal lymphocytic infiltration in the liver. The kidneys were normal. The atheromatous lesion in the aorta consisted of plaques which were distributed throughout the aorta. Lesion belonged to grade II (++). Intimal plaques showed fatty infiltration, subintimal haemorrhage and hyalinization (fig.7,8,9). Myocardium also showed a fatty change.

Table 15 shows enzyme activities in liver and serum of different groups. Due to small number of animals in each group with high biological variation

Fig.6 : Normal Aorta of Rabbits.

Fig.7 : Aorta of drug treated cholesterol fed rabbit showing extensive atheromatous plaques.

Fig.8: A strip of aorta of drug treated cholesterol fed rabbit showing large muscular layer with superimposed fatty plaque showing lipid laden vacuolated cells under the endothelium.

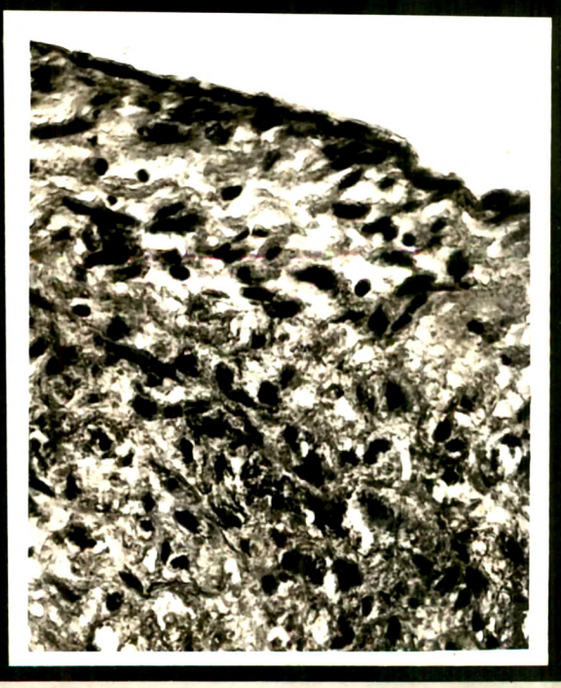

Fig.9: Section of aorta of drug treated cholesterol fed rabbits showing intact endothelium and subendothelial large plaque showing lipid laden cells and gruelly material which is likely to ulcerate at the surface.

TABLE 15

* Enzyme activities in liver and serum of insulin/tolbutamide (with or without cholesterol feeding) treated rabbits.

Enzyme	Control ← I	Insulin Set 'A' II	Tolbutamide → III	Cholesterol IV	Cholesterol + Insulin Set 'B' V	Cholesterol + Tolbutamide → VI
Liver G-6-P-D-	21.40 ± 7.26 (5)	18.94 ± 8.53 (4)	20.56 ± 2.86 (5)	30.76 ± 13.7 (5)	30.20 ± 18.45 (4)	25.65 ± 5.75 (5)
Malic Enzyme	53.30 ± 13.42 (5)	75.49 ± 17.96 (5)	44.79 ± 6.61 (5)	62.74 ± 13.64 (5)	56.18 ± 23.82 (5)	62.83 ± 17.27 (5)
Citrate Cleavage	40.30 ± 12.57 (5)	45.56 ± 26.30 (5)	46.50 ± 29.1 (5)	52.32 ± 15.94 (5)	51.96 ± 23.40 (5)	75.18 ± 34.16 (5)
Lactic dehydrogenase	1914 ± 927 (6)	1846 ± 947 (5)	1483 ± 658 (5)	2108 ± 630 (5)	2191 ± 852 (5)	1904 ± 721 (6)
Pyruvate[1] Kinase	121.47 ± 41.4 (5)	266.75 ± 102.8 (5)	198.95 ± 57.6 (5)	253.14 ± 80.37 (5)	272.17 ± 28.8 (5)	224.65 ± 62.75 (5)
Serum G.O.T.[2]	126.6 ± 37.2 (6)	120.0 ± 14.4 (5)	150.0 ± 69.0 (6)	115.0 ± 65.4 (5)	156.0 ± 59.0 (5)	250.0 ± 132.0 (6)
Serum G.P.T.	113.67 ± 48.3 (6)	240.0 ± 71.1 (4)	88.0 ± 27.8 (5)	144.0 ± 37.1 (5)	185.0 ± 62.5 (4)	164.0 ± 45.3 (5)

* Values are means ± S.D. Figures in the parenthesis indicate number of observations. One unit (Wroblewsky Unit) is the amount of enzyme which changes the O.D. by 0.001 at 340 mu/minute in a 3 ml of assay mixture at 24°C.

1. One Unit is the amount of pyruvic acid/100 mg of protein/minute.
2. For G.O.T. and G.P.T. one unit is the amount of pyruvic acid/mg of protein/hour.

and short duration of treatment, statistically significant difference could not be found in many cases.

It can be noted that there was no significant difference in the Glucose-6-phosphatedehydrogenase activity of liver between the groups as shown in table 16. In case of malic enzyme, insulin group showed higher levels of malic enzyme than the control group but it however, just failed to achieve statistically significant difference. Tolbutamide group did not show any difference in malic enzyme activity as compared to control group. But insulin group showed significant difference in malic enzyme activity as compared to tolbutamide group ($P < 0.01$). In cholesterol fed groups, there was no significant difference in the activity of malic enzyme in all the groups as seen in table 16.

Citrate cleavage enzyme activity showed no significant difference in any of the groups. Lactic acid dehydrogenase, pyruvate kinase and serum glutamate oxaloacetate transaminase activities were also found to show no statistically significant difference. However, serum glutamate pyruvate transaminase (SGPT) activity was significantly higher in case of insulin

TABLE 16

Table showing 't' and 'p' values for comparisons between various groups for liver G-6-P-D, malic enzyme, citrate cleavage, lactic dehydrogenase, pyruvate kinase, serum G.O.T. and G.P.T.

Group	G-6-P-D		Liver malic enzyme		Citrate Cleavage		Lactic dehydrogenase		Pyruvate Kinase		Serum SGOT		Serum SGPT	
	't'	'p'	't'	'p'	't'	'p'	't'	'p'	't'	'p'	't'	'p'	't'	'p'
Set 'A'														
I VS II	0.464	-	2.219	-	-0.405	-	0.120	-	-0.485	-	0.375	-	-3.380	P< 0.01
I VS II	0.240	-	1.270	-	-0.443	-	0.875	-	-0.775	-	0.731	-	1.050	-
II VS III	0.405	-	3.569	P<0.01	-0.052	-	0.700	-	-0.407	-	0.967	-	4.432	P< 0.005
Set 'B'														
IV VS V	0.052	-	0.545	-	0.029	-	-0.165	-	-0.500	-	1.093	-	1.261	-
IV VS VI	0.774	-	0.090	-	-1.325	-	0.500	-	0.623	-	-2.093	-	0.741	-
V VS VI	0.529	-	0.500	-	-1.257	-	0.500	-	-1.537	-	-1.478	-	0.509	-

't' value denotes calculated values of 't'. 'p' value indicates probability.

P values are given only when the difference is significant.

group than in control group ($P < 0.01$). Insulin group also showed significantly higher activity of SGPT as compared to tolbutamide group ($P < 0.005$). Comparison of all other groups with their respective controls and comparison within the treatments did not show statistically significant difference in SGPT activity.

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