INTERRELATIONSHIP BETWEEN R. Q., BLOOD SUGAR AND BLOOD PYRUVATE LEVELS AFTER GLUCOSE LOADING IN YOUNG HEALTHY SUBJECTS

By

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A preliminary study conducted on 18 young healthy subjects showed three distinct patterns of R. Q. changes during normal glucose tolerance test of 2 hours duration. Subjects were grouped into three categories viz., A, B and C according to their R. Q. reaction pattern. When the three categories of subjects were administered Prednisolone (once only) and hypoglycaemic drugs (two types) for a period of 7 days at suitable intervals, the R. Q. and the blood pyruvate levels showed distinctive changes in each of the three groups following glucose loading. In contrast, the blood glucose levels of each category followed more or less a similar pattern without much variations, compared to their original basal G. T. T. values.

The possible causes of changes in R. Q. and blood pyruvate levels vis-a-vis blood glucose concentration both before and after the administration of pharmacological agents and their possible significance have been discussed.

Introduction

Conflicting opinions are frequently expressed by the specialists on the issue of a pilot's fitness to fly, when an abnormality in G.T.T. is detected during routine investigations. Differences of opinions arise mostly due to different interpretations of the results of G.T.T. in absence of corroborative findings. In this respect "border line" G.T.T. abnormalities have always posed a problem to the specialists.

In absence of a clear evidence, it is difficult to predict the course of diabetes specially at the early and latent stages. Quite a large percentage of latent diabetes either become permanently diabetic or revert back to normal with or without treatment. Abnormality in G.T.T. may be due to various causes. Chief disorder in diabetes is with carbohydrate metabolism. As the disease advances, all other metabolisms are gradually affected at one stage or the other. Glucose tolerance test reflects only the sum total result of glucose metabolism and does not furnish any information of the intermediary stages. Insufficient knowledge of intermediary metabolism of glucose is the chief contributory cause of controversy since, the interpretations of the results

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based on G.T.T. findings alone is not dependable for diagnostic purposes for many reasons.

The most important aspect of glucose metabolism is the capacity of the cells to utilise glucose in a judicious manner. Blood sugar level at any moment reflects only the balance of glucose left in the blood after its conversion to fat, deposition as glycogen and its utilisation by the

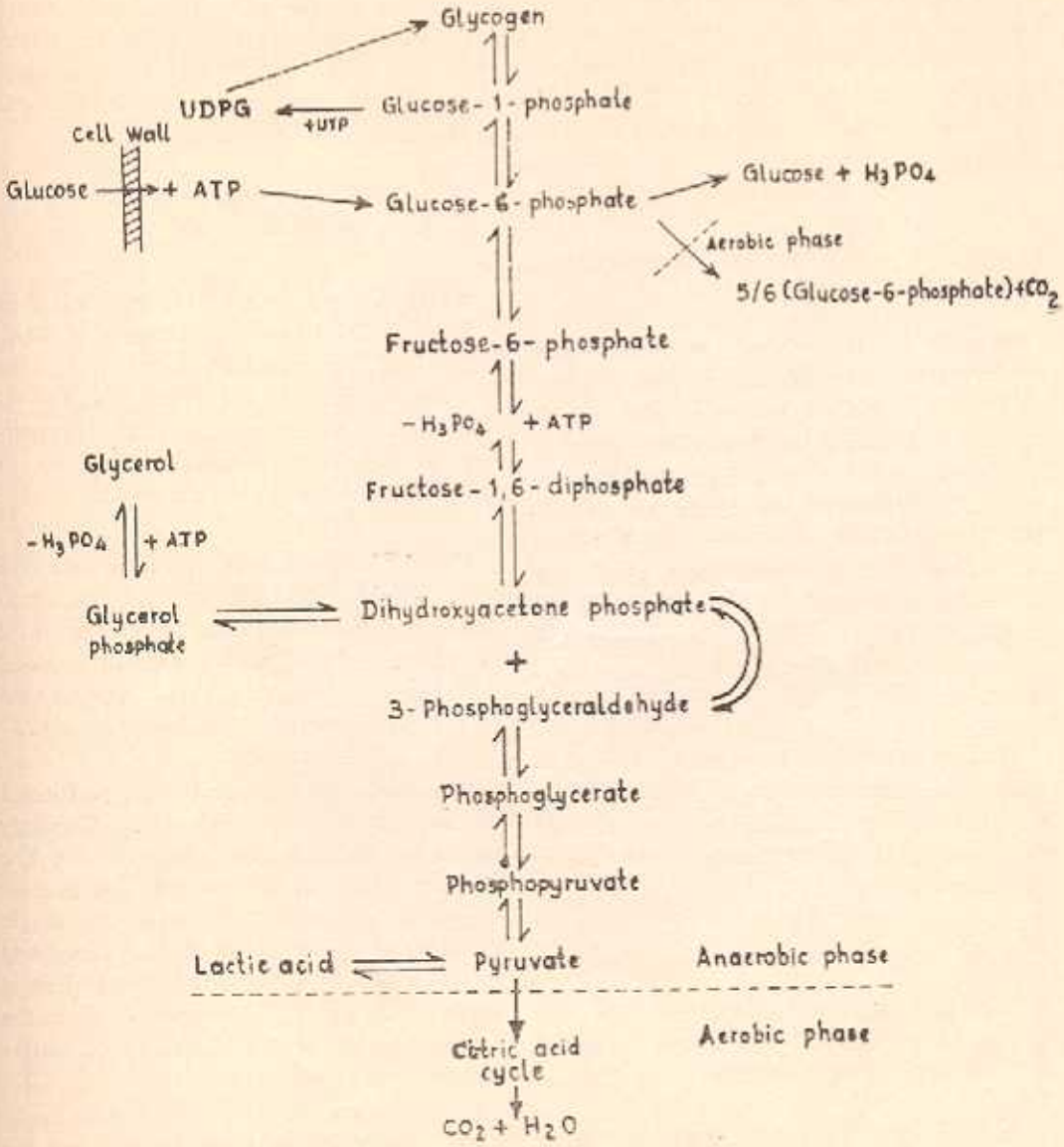


FIG. 1- PATH-WAYS OF CARBOHYDRATE METABOLISM.

cells. These physiological processes are all regulated by various enzyme activities under the influence of endocrine secretions. In diabetes, any one of these or more than one, may be at fault, which may be of temporary or permanent nature depending upon the site and the degree of the disorder in the whole metabolic chain. Blood sugar values alone do not furnish any one of the informations cited above, which is so essential for the purpose of a reliable diagnosis. (Fig. 1) Inspite of this inadequacy, glucose tolerance test, continues to be the sheet anchor for diagnosis and prognosis of diabetes of all descriptions.

In view of the above, a preliminary study was conducted on young healthy subjects with a view to explore the possibilities of eliciting the behaviour of some vital links in the intermediary metabolism, which are reflected on 'easy to investi gate' biochemical reactions. Such informations are likely to provide a vital clue as to the nature of the disorder even in latent or early stages of diabetes, for which further studies are being contemplated.

Studies conducted on some of the physiological parameters on 18 subjects indicate the above possibilities. The results of these preliminary studies are being presented here.

Method

Eighteen healthy subjects in the age group between 20-30 years were selected for the purpose of the study.

Respiratory Quotient (R.Q) for each subject was determined at the fasting level, and after 1 and 2 hours of glucose administration, whenever it was required to be estimated. For this purpose, subjects after adequate rest in bed, were made to breathe through a mouth-piece connected to a Douglas bag. They breathed in atmospheric air and breathed out into the Douglas bag for 6 minutes. The expired air was analysed in a Scholander micro gas analysis apparatus in order to determine the CO_2 output and O_2 consumption. R. Q. was estimated from these values using the formula :

$$R.Q. = \frac{Ce - 0.03}{0.265\ Ne - Oe}$$

where Ce represents CO_2 percentage in expired air ; 0.03, the percentage of CO_2 present in the inspired air ; 0.265, the correction factor for the volume of inspired and expired air ; Ne, the percentage of N_2 in the expired air ; Oe, the percentage of O_2 in the expired air.

For the estimation of blood pyruvic acid at fasting level and 1 hour after the administration of the test dose of glucose, 1 ml of venous blood was collected. Blood pyruvic acid estimation was done by the method recommended by Joiner et al[1].

Glucose tolerance test was performed by Somogyi-Nelson[2] method. Capillary blood was collected from finger prick. The subjects were given 100 gms. of glucose dissolved in water orally in a suitable concentration as a test dose. Blood and urine samples were collected at fasting stage and every 30 minutes after the administration of glucose till 2 hours samples were collected.

All the subjects were investigated for R. Q., blood pyruvic acid and blood sugar on three different occasions at suitably spaced intervals in the following manner :-

Table 1. Basal R.Q. values of the subjects in groups A, B and C before and after glucose loading.

GROUP	SUBJECT No.	R.Q.		
		Fasting	1 hr. after glucose	2 hrs. after glucose
A	1	0·82	0·99	0·94
	2	0·83	0·92	0·90
	3	0·79	0·90	0·85
	6	0·81	0·87	0·87
	11	0·83	0·87	0·83
	12	0·74	0·82	0·79
	13	0·75	0·83	0·80
	15	0·80	0·81	0·81
	Mean	0·80	0·88	0·85
B	5	0·79	0·78	0·82
	9	1·09	0·99	1·00
	14	0·94	0·93	0·96
	18	0·94	0·89	0·95
	Mean	0·94	0·90	0·93
C	4	0·88	0·92	0·95
	7	0·77	0·83	0·85
	8	0·80	0·98	1·00
	10	0·82	0·85	0·92
	16	0·76	0·80	0·83
	19	0·74	0·81	0·83
	Mean	0·80	0·87	0·90

(a) Basal — i. e. when no pharmacological agents were administered to the subjects. The findings of this series served as a reference.

(b) After the administration of Prednisolone.

(c) After 7 days of administration of either Rastinon or DBI, three tablets a day.

On all occasions G.T.T. and R. Q. determination were conducted upto 2 hours.

Prednisolone primed G.T.T. was performed on the next day of the basal readings. 10 mg. of prednisolone were administered at 8 hours interval (2 doses) in the Air Force Hospital Bangalore prior to the test, the last dose was given 2 hours before the test.

Subsequently, the subjects were put at random either on Rastinon or DBI for a period of 7 days, without the knowledge of the experimenters as a precaution against experimental bias.

Results and Discussion

Basal R.Q. values of all the 18 subjects are given in Table 1. Subsequent to the determinations of R.Q. values, subjects were divided into three groups viz., A, B and C on the basis of the pattern of R.Q. changes from the fasting level after glucose administration. This practice was followed with a view to ensure uniformity among the groups and for subsequent comparison of intra and inter group experimental results. According to this classification, out of the 18 subjects, 8 were bracketed in group A, 4 in group B, and 6 in group C.

Group A represented the subjects who showed a rise in R.Q. to a higher value from the fasting level at 1 hour, followed by a fall at 2 hours after glucose administration.

Group B subjects showed a fall of R. Q. at 1 hour followed by a subsequent rise at 2 hours after glucose administration.

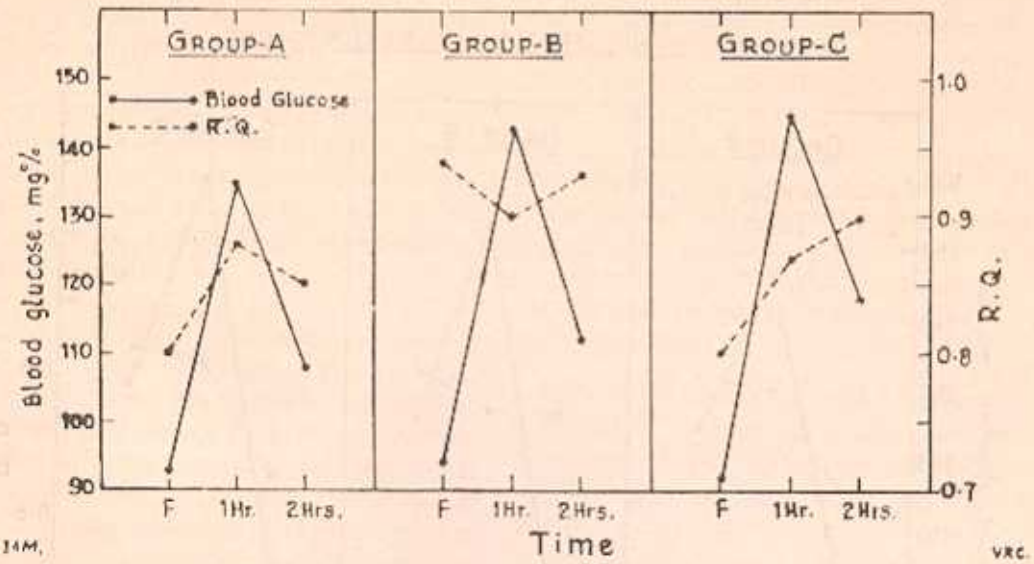
Group C represented the subjects who showed a continuous rise of R.Q. from the fasting level upto 2 hours after glucose administration.

There was one subject who showed a continuous fall of R.Q. through all the phases after glucose administration. This subject has not been included in the list, in view of its being an isolated case. However, in a proposed future study this type of reaction will be kept in view and analysed.

Relative changes of R.Q. and blood glucose level are shown in Fig. 2. It will be noted, that there were minor inter group variations in the peak and 2 hours values of blood glucose. In contrast, there were considerable differences in the pattern of R. Q. between A, B and C.

In a molecular structure, the relative quantities of oxygen and carbon contained in the three categories of biological "fuel" differ. Therefore the relative volumes of CO_2 produced and O_2 consumed during the metabolism of each type of food also vary. R.Q. values therefore, give an indication as to the type of 'fuel' being utilised by the cells during the metabolic processes. Cathcart and Markowitz[3] are of the view that R.Q. in a given instance is a resultant of several different metabolic proce-

FIG. 2 AVERAGE BASAL VALUES OF R.Q. AND BLOOD SUGAR IN GROUPS A, B AND C BEFORE AND AFTER GLUCOSE LOADING.



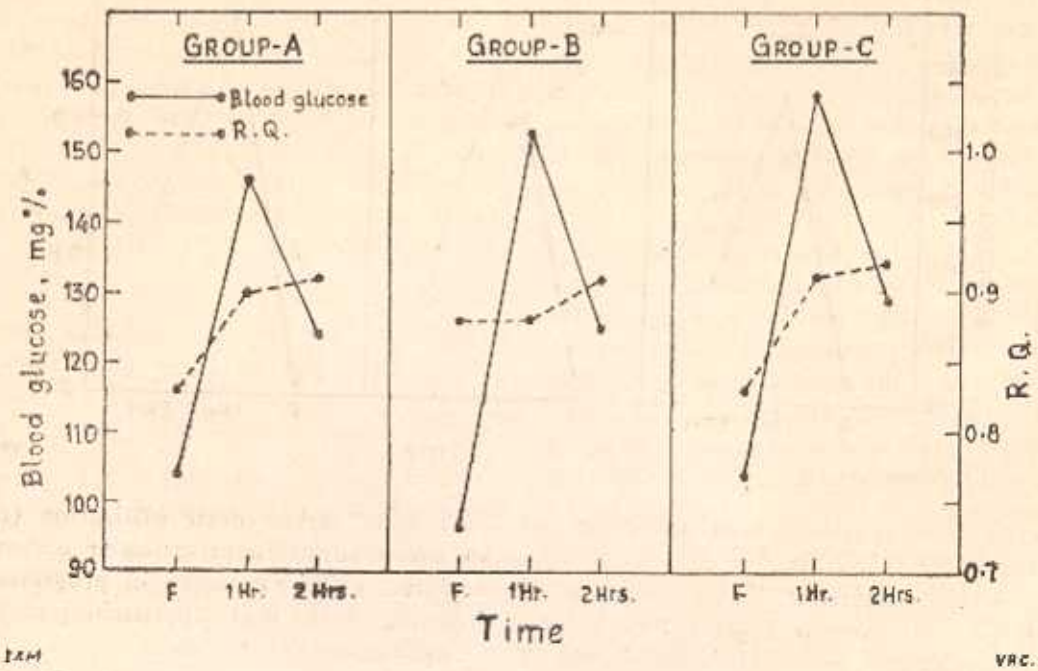
sses, viz., synthesis, interconversion and combustion.

While discussing the significance of R.Q. in relation to general metabolism, Cantarow and Schepartz[4] stated that, if the organism is utilising exclusively carbohydrate, the R.Q. would be 1.0, if utilising fat alone, R.Q. would be 0.7. and if protein alone the R.Q. would be 0.8. Conversion of carbohydrate to fat results in R.Q. values increasing above 1.0 because some of the oxygen of the carbohydrate molecule, which contains relatively more of this element as compared to fat, becomes available during the oxidative process of its conversion resulting in less requirement of O_2 in the inspired air for the organism. On the same analogy conversion of fat to carbohydrate would be reflected on the R,Q. value being less than 0.7 and for protein the R.Q. values will range between 0.632 – 0.726. According to them a progressive decrease in the proportion of carbohydrate utilisation (i.e. in carbohydrate deprivation or diabetes mellitus) will be reflected in progressive lowering of the R.Q. approaching to 0.7 or even below this value.

On the above basis, it may be said that the three groups of subjects A, B and C in the present experiment, reacted differently after the administration of glucose perhaps because their basic enzymatic reaction patterns were different.

Fig. 3 shows how the R.Q. and blood glucose values reacted before and after glucose loading following the administration of Prednisolone. There were no significant intra group changes in blood glucose values as compared to their basal values as shown in Fig. 2. Blood glucose value wise, all the subjects in all the groups reacted in a similar manner. However the changes in the R.Q. values

FIG. 3 AVERAGE R.Q. AND BLOOD SUGAR VALUES IN THE GROUPS A, B AND C BEFORE AND AFTER GLUCOSE LOADING FOLLOWING ADMINISTRATION OF PREDNISOLONE.



after Prednisolone administration are different in the three groups.

In group A, the R. Q. values showed a continuous rise (Fig 3) after Prednisolone instead of "first rise then a fall" pattern seen earlier in basal condition.

In group B, the R. Q. values showed no rise from fasting 1 hour after Prednisolone therapy. It rose subsequently at 2 hours (Fig. 3) which is at variance with the basal pattern as seen in Fig. 2.

Group C subjects differed from the other groups in this respect. Even after Prednisolone administration they did not change their R. Q. response pattern from the original i. e. "a continuous rise" (Fig. 2 and 3) type remained unaltered.

It is known, that glucocorticoids by stimulating neoglucogenesis tend to "push up" the blood glucose level during the G. T. T. Originally cortisone glucose tolerance test (C. G. T. T.) was introduced by Fajans and Conn[5] with a view to "unmask" the potential diabetics i. e. those apparently normal but destined to become frankly diabetic later. According to the criteria laid down by them (using venous blood and true glucose measurements) the limits of normal are taken as 160 mg./100ml. at 1 hour, 150mg./100ml. at 1½ hours and 140 mg./100 ml. at 2 hours. The usefulness of this test is still not acceptable to many. Pyke[6] is of the opinion that the test is likely to be no more reliable than other tests in order to differentiate normals from the diabetics.

Whatever its worth as a test, the significant point to note in the present experimental set up is that there is a significant trend of changes in R. Q. values in groups A and B and less significantly in group C (Fig. 3) after Prednisolone administration. These are indicative of the influence of Prednisolone on the metabolism in groups A and B and not in Group C. In other words the metabolism of Group C is not influenced by Prednisolone in contrast to Groups A and B.

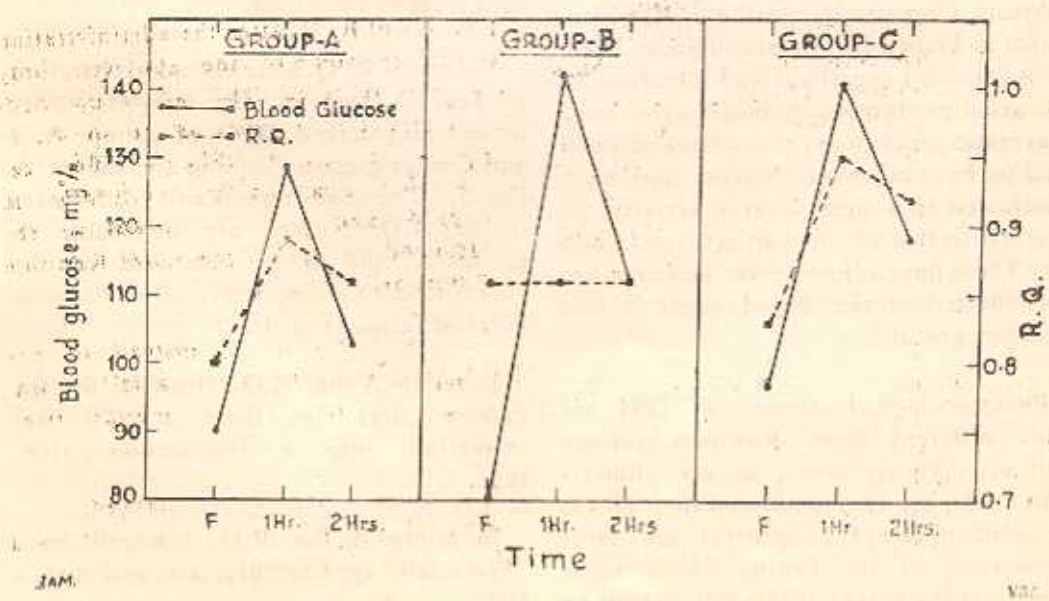
Does it indicate then; that the people who are considered normal on the basis of G.T.T may have different metabolic mechanisms keeping their blood glucose level within the normal limits? At least the inter group differences of R.Q. at "basal" state and intra group differences after Prednisolone administration point to this fact.

Since R.Q. in a given instance is the resultant of several metabolic processes as pointed out by Cathcart and Markowitz (*ibid*) as opposed to blood glucose level which merely reflects the balance of the whole process, it appears from the above results, that the R.Q. gives a better indication of the adjustments that take place in the whole metabolic chain, after glucose loading than the blood sugar values alone. A close look at the pathways of carbohydrate metabolism (Fig. 1) and the results obtained indicate such a possibility.

Further evidence in this respect is provided by the behaviour of R.Q. and blood glucose levels after the administration of hypoglycaemic drugs of known pharmacological actions.

It will be seen from Fig. 4, that after the administration of Rastinon (500 mg)

FIG. 4 AVERAGE R.Q. AND BLOOD SUGAR VALUES IN THE GROUPS A, B AND C BEFORE AND AFTER GLUCOSE LOADING FOLLOWING ADMINISTRATION OF RASTINON FOR SEVEN DAYS.



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thrice daily for 7 days, some of the subjects of group A (only those who received Rastinon in Group A) showed a similar pattern of R.Q. changes as seen earlier during "basal" state (Fig.2). It was not so with group B.

In group B the R.Q. remained unaltered all through the period of investigation upto 2 hours. This reaction cannot be explained on the basis of known physiological facts.

In group C, however, Rastinon produced a different reaction altogether as compared to "basal" and after Prednisolone, administration. After the administration of Prednisolone, the R.Q. changes observed were very similar to those of 'basal' conditions, where as after Rastinon administration (Fig. 4), the basal pattern of R.Q. i.e. "a continuous rise" type was converted into a "first rise and then a fall" which was not seen earlier in group C subjects.

It, therefore, indicates that in group C subjects, after administration of Rastinon, which is known for its stimulating effect on B cells of Langerhans and therefore of increased production of insulin, the basic enzymatic processes of glucose metabolism (and perhaps other metabolism too) were readjusted to a new level of activity, in contrast to that of the subjects in Group A. These finer adjustments. however, are not reflected on the blood sugar values of either groups.

Pharmacological actions of DBI are quite different from Rastinon, though both are hypoglycaemic agents. Butterfield et al[7] are of the opinion that effects of sulphonylureas in general are most pronounced on the fasting blood sugar level, but the extent of this fall cannot be taken as a practical guide for treatment. It has been found by many workers notably by Duncan et al[8], that the lower level of fasting blood sugar during treatment is not associated with any marked changes in the mean rise or fall of blood sugar during standard G.T.T. That the effects of sulphonylureas as hypoglycaemic agents is due to the increase in B cells activity, has been proved by the electron microscopic studies conducted by Volk et al[9] and many others.

Experimentally DBI has been found to reduce tissue respiration and produce lactacidosis. This was confirmed by the work of Tranquada et al[10] and others.

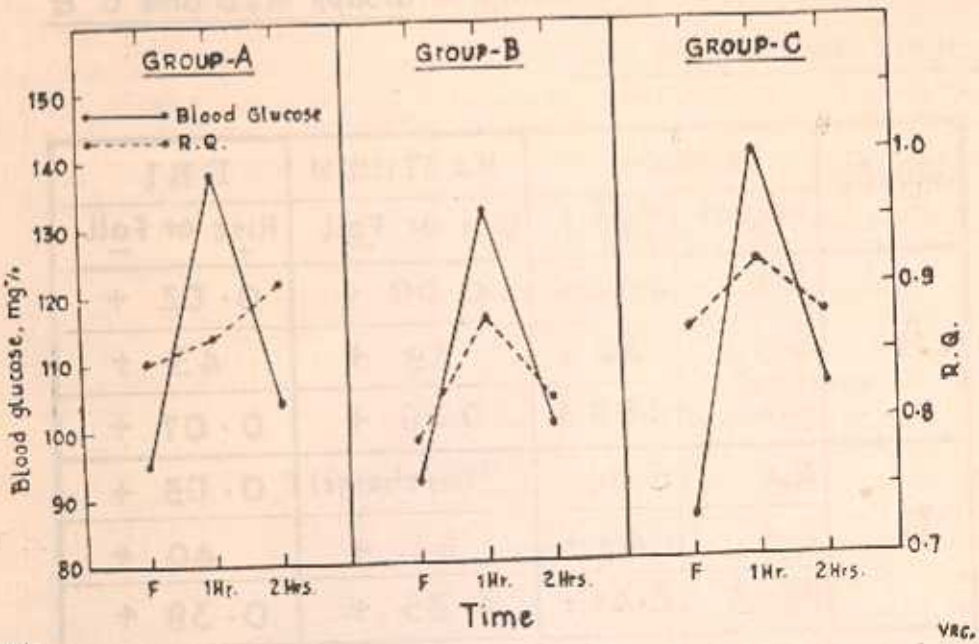
Therefore from the above, it is expected that DBI administration is likely to enhance anaerobic cycle of Embden Meyerhof pathway of glucose metabolism in contrast to Rastinon. The effects of these two drugs logically therefore should be reflected on O_2 consumption and pyruvic acid level of the blood.

Values of R. Q. after the administration of DBI in some of the subjects (only those who received DBI) of group A, B and C after glucose loading are shown in Fig. 5. The most remarkable differences in the R.Q. pattern are noticeable in groups A and B as compared to the corresponding changes seen after Rastinon administration (Fig. 4).

In group A the R.Q., instead of the pattern "first rise, then a fall" was converted into a "continuous rise" type.

In group B the R.Q. changed from "first a fall" type to "first rise and then a fall".

FIG. 5 AVERAGE R.Q. AND BLOOD SUGAR VALUES IN THE GROUPS A, B AND C BEFORE AND AFTER GLUCOSE LOADING FOLLOWING ADMINISTRATION OF DBI FOR SEVEN DAYS.



The R.Q. changes in group C after DBI administration was lesser than the changes observed after Rastinon. But the group reaction of "continuous rise" was converted into "first rise then a fall" after both Rastinon and DBI.

The above results therefore confirmed that the pharmacological effect of DBI on glucose metabolism is significantly different from that of Rastinon, a fact which is well reflected on the R.Q. values and not so much on blood sugar values.

Fig. 6 depicts the average blood pyruvic acid levels at fasting and at 1 hour after glucose loading in basal condition and following Rastinon and DBI therapy. The maximum rise of pyruvic acid in blood from fasting to 1 hour was noticed after Rastnon therapy in group C and the minimum was noticed after DBI therapy in Group A. The R. Q. changes for the corresponding groups also ran parallel to that of pyruvic acid levels i. e. maximum rise after Rastinon therapy in Group C and minimum rise after DBI therapy in group A (Table 2). The interpretation of these results will have to wait till larger number of samples both from normal and diabetic subjects are collected.

Out of the 18 subjects studied, subject Nos. 8 and 10 showed borderline abnormalities in basal GTT whereas subject No. 19 showed a lag storage type of GTT Curve (Table 3). All the three subjects belonged to group C according to R. Q.

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changes of R.Q., Blood Sugar and Pyruvic Acid from Fasting to 1-hour after glucose loading in Groups A, B and C & subject Nos. 8 and 10.

Table - 2

GROUPS	BASAL		RASTINON	DBI
	Values of	Rise + Fall -	Rise or Fall +	Rise or Fall +
A	R.Q.	0·08 +	0·09 +	0·02 +
	B.G.	42 +	39 +	43 +
	Pyr.A	0·38 +	0·26 +	0·07 +
B	R.Q.	0·04 -	±(No change)	0·03 +
	B.G.	49 +	62 +	40 +
	Pyr.A	0·45 +	0·35 +	0·38 +
C	R.Q.	0·07 +	0·12 +	0·05 +
	B.G.	53 +	44 +	54 +
	Pyr.A	0·35 +	0·44 +	0·30 +
Subject No.8	R.Q.	0·18 +	0·16 +	
	B.G.	60 +	50 +	
	Pyr.A	0·87 +	0·64 +	
Subject No.10	R.Q.	0·03 +	0·06 +	
	B.G	60 +	60 +	
	Pyr.A	0·25 +	0·23 +	

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