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THE ACTION OF KETOSIS ON CARBOHYDRATE METABOLISM.

By

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PREFACE

This work was carried out in the Medical Wards of the Royal Hospital for Sick Children, Glasgow and before submitting this thesis it is my very pleasant task to acknowledge my indebtedness to Professor Leonard Findlay at whose suggestion this investigation was begun and under whose help and guidance it was carried out. I offer to him my most sincere thanks. To Dr. M.Morris, of the Biochemical Department, I also owe a debt of gratitude for his encouragement and the help received regarding the technical side of the work.

The investigations were carried out during the tenures of the Muirhead and the McCunn Medical Research Scholarships.

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Introduction.

It has long been known that disturbance in carbohydrate metabolism frequently leads to an appearance of ketone bodies in the urine. This has usually been associated with a faulty intermediate oxidation of fats and 'ketosis', indeed, is taken as denoting an appearance of abnormal fatty acids in the blood. In the domain of disease in infancy and childhood there is frequently met the condition of acetonuria, until recently, wrongly considered pathognomonic of acidosis. This condition of acetonuria, or better, ketosis, has been attributed to a defect in carbohydrate metabolism, or as Shaffer[1] has put it, "carbohydrate starvation", and has been treated by administration of glucose. During the course of an investigation into the phenomena of ketosis in childhood, it was found that there was a definite disturbance in carbohydrate metabolism, suggesting that the symptoms and biochemical manifestations associated with ketosis may be the result of a primary upset in the intermediate metabolism of fat, with the carbohydrate disturbance as a secondary occurrence.

The work detailed in the present thesis was undertaken with the view of throwing more light on this problem. Early in the course of this investigation it was found that glycosuria was frequently manifest in conditions of ketosis and/

and acidosis. It was therefore necessary to have some standard of the normal and so the normal tolerance for glucose and the renal threshold in healthy children were investigated.

The first two sections are devoted to a consideration of

(1) The Glucose Tolerance Test
and (2) The Renal Threshold for Glucose.

In the third section the effect of Ketosis on the blood sugar curve is considered together with a study of its influence on the action of those endocrine substances which are known to be of importance in carbohydrate metabolism, namely adrenalin, insulin and pituitrin. This section concludes with an investigation of liver function in ketosis by means of the laevulose test.

As the condition of ketosis produced in this series of investigations is associated with an acidosis, as was shown by Brown and Graham,(2) it was therefore necessary to determine whether the results obtained were due to the acidosis per se and not to the effects of the ketone bodies. In section four carbohydrate metabolism is studied in an acidosis produced by Ammonium Chloride which is not associated with a ketosis.

Section five contains the report of a preliminary series of investigations into the histological changes produced by acetone with special reference to the glycogen content of the/

the liver. In the concluding section, six, the general findings are briefly discussed and an attempt is made to draw conclusions based on these findings.

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SECTION I.

Glucose Tolerance Tests.

Within recent years glucose tolerance has been used as a means of investigating disturbances of carbohydrate metabolism, especially when following disorders of the endocrine system. In such tests, it has generally been accepted that a normal adult of 60 kgrm weight can ingest and metabolise 100 grm. of glucose without the appearance of glycosuria. The ability to deal with more than this amount is considered evidence of an increased carbohydrate tolerance, and the presence of glycosuria following the ingestion of less than 100 grm. points to a decreased tolerance. In the case of children it has been customary to consider that there is an increased carbohydrate tolerance if more than 2 grm. of glucose per kgrm of body weight can be ingested without the appearance of glycosuria.

This view of carbohydrate tolerance has not, however, met with universal acceptance. Samson Wright (3) says, "It is difficult to understand the term 'increased sugar tolerance' which is so frequently used, since nausea develops before the limits of ingestion are reached". He also states that a certain proportion of subjects develop very slight glycosuria with 300-500 grm. of glucose. Taylor and Hulton (4) consider/

consider that in the majority of healthy adults there is no limit to the assimilation of glucose, but a survey of their findings shows that in 6 of their 25 cases glycosuria appeared with 200 grm. Gray,[5] in his paper on "Blood Sugar Standards" points out that of 129 apparently normal persons 40% showed glycosuria after the ingestion of 100 grm. of glucose and Goto and Kuno[6] also note glycosuria after a similar amount in 62% of their cases. Benedict and Osterberg[7] report glycosuria in two cases after 40 and 60 grm. of glucose respectively.

Details of the investigation of the glucose tolerance of 32 children, in whom there was no reason to suspect any disturbance of carbohydrate metabolism, are given below. The children varied in age from 1½ to 12 years and the majority had been suffering from chorea, but there were also 5 patients convalescent from rheumatic arthritis, 2 suffering from taeniasis and one case eadh of rheumatic pericarditis, convalescent pneumonia, chronic pneumonia, congenital syphilis, chloroma and rickets. The children with chorea and those suffering from rheumatic infections were receiving daily 90 grains of Sodium Salicylate and 180 grains of Sodium Bicarbonate. These drugs were, however, omitted during the day of the test.

The test was discontinued when the child was actively sick/

sick with an amount of glucose larger by 1 grm per kgrm. of body weight than that tabulated. Seven of the children could not be induced to take more glucose than that recorded because of the fear that they would be sick. The glucose, in amounts varying from 1 to 11 grm. per kgrm. of body weight was given in place of breakfast after an all night fast. The urine was collected for 4 hours at hourly intervals and tested for Sugar with Fehlings Solution. When there existed any doubt about the result the phenyl-hydrazine test was also carried out.

The results have been tabulated in Tables I and II.

Table I.

Cases in which Glycosuria Occurred.

Case	Sex	Age Yrs	Weight Kilos.	Glucose per Kgrm. of Body weight	Amount of Glucose Grm.	Disease	Sugar in urine
A.H.	M	10½	22	1.0	22	Chorea	Trace
D.T.	M	10	25	4.0	105	"	+
J.C.	F	5	17.5	5	88	Rheumatism	+
E.McC	F	8	20	6	121	Chorea	Trace

Table I gives the details of the four cases in whom glycosuria was produced and of these A.H. is the only one whose sugar tolerance can be said to be markedly low. The patient/

patient was examined daily for glycosuria for a period of two months, but the tests were always negative and he never showed any symptoms of diabetes. The blood sugar curve after 22 grm. of glucose reached a value of .208% after 1 hour but fell below the fasting level in 2 hours. He cannot be said to be a "renal glycosuric" since his threshold was found to lie above the level of 184%. The tolerance in the other three cases was broken with quantities of glucose equal to 4, 5 and 6 grm. of glucose per kgrm. of body weight respectively.

All but one of the cases in Table II were able to take more than 2 grm. of glucose per kgrm. of body weight without either nausea or the appearance of sugar in the urine and 20 of the 32 children were able to ingest a total of 100 grm. or more, irrespective of age or weight, without glycosuria. The amount of glucose which children can take, without nausea or vomiting, varies enormously, and seems to have little or no relationship to age, weight or sex. The quantity varied from 30 to 270 grm. One child of 3 years took a total of 110 grm of glucose (i.e. 11 grm. per kgrm. of body weight) and another of 10½ years was able to take 67 grm. only (i,e, 2.5 grm. per kgrm. of body weight) without nausea and sickness being produced. This fact (that of nausea) complicates any conclusions which may be drawn regarding glucose tolerance.

Twenty/

Table II

Case	Sex	Age yrs	Weight Kilos.	Glucose per Kgrm. Body Weight	Amount of Glucose grm	Disease
J.W.	M	4½	15	2 grm.	30	Chronic Pneumonia
J.McL	M	10½	27	2.5 "	67	Rheumatism
E.C.	F	5½	16.5	3 "	49	Pericarditis
F.W.	F	2 11/12	11.8	3 "	36	Conval. Pneumonia
J.F.	M	12	27	3.5 "	100	Severe Chorea
N.G.	F	10	26	3.5 "	100	" "
M.H.	F	11	28	3.5 "	100	V. Slight Chorea
S.M.	M	11½	25.5	4 "	105	Severe Chorea
W.D.	M	10	24	4 "	100	Tape worm
J.M.	F	1½	8	4 "	33	Chloroma
M.M.	F	9	19.5	4 "	80	Chorea
M.F.	F	7	19	4 "	76	Chorea
F.F.	M	9	25	4 "	100	Chorea
M.F.	F	10	23	4 "	100	Chorea
J.H.	M	10½	28	4 "	112	Chorea
A.McK	F	12	24	4 "	100	Tape worm
G.McD	F	12	26	4 "	105	Chorea
J.McL	F	10	21	4.5 "	100	Chorea
J.M.	M	7	19	4.5 "	90	Chorea
J.G.	M	11	28	5 "	140	Chorea
M.T.	M	12	23	6 "	138	Chorea
G.G.	M	6½	20	6 "	120	Chorea
J.F.	M	9 2/3	21	6.4 "	145	Rheumatic Fever
R.H.	M	6 1/3	16	7 "	112	Rheumatic Fever
M.B.	F	5½	16	8 "	128	Neuro-Syphilis
R.McG	F	5 11/12	15	8 "	120	Chorea
R.McN	M	11	27	10 "	270	Rheumatism
W.K.	M	3 3/12	10	11 "	110	Rickets.

The Wt. in Kilos and the Amount of Glucose are only approximate to nearest Kilo and grm.

Twenty-three of these thirty-two children were able to take 4 grm. of glucose per kgrm. of body weight without glycosuria and only in two of the thirty-two cases was the sugar tolerance broken with this or a less amount.

It may therefore be generally concluded that the majority of normal children are able to ingest an amount of glucose equal to 4 grms. (at least) per kgrm. of body weight without glycosuria resulting. The term "an increased sugar tolerance" cannot therefore be used with any meaning since, normally, nausea probably develops before the limits of ingestion are reached.

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SECTION II.

The Blood Sugar Curve and Renal Threshold.

The effect of a large quantity of glucose on the blood sugar curve of ten apparently normal children was studied. Eight of the children were recovering from an attack of chorea, one, case VI, was convalescent from rheumatic arthritis and another, case V, had recently been treated for tapeworm, but during the period of study there was no sign of segments or ova in the faeces. All the children with one exception (case V) were receiving 90 grains of Sodium Salicylate and 180 grains of Sodium Bicarbonate daily, but this was omitted on the day of the test. On one occasion, by inadvertence, a child received 15 grains of Sodium Salicylate and 30 grains of Sodium Bicarbonate immediately after the glucose had been given. The blood sugar curve obtained on that occasion did not differ in any marked degree from that obtained when the test was repeated with glucose alone. During the period of study the children were confined to bed and received the routine mixed diet of the hospital. One grm. of glucose per kgrm. of body weight was given for the first test, and, some days later, for the second test, 100 grm. of glucose except in cases IX and X where 121 and 88 grm. were given respectively/

respectively. MacLean's[8] method was used, as throughout in this work, to estimate the blood sugar values. The urine was collected where possible at half hourly intervals immediately after the withdrawal of the specimen of blood, and tested for the presence of sugar and acetone.

The results are tabulated in Table III.

The generally accepted normal fasting blood sugar value in the adult lies between .070% and .120% with an average value of .100%. Bass[9] found in a series of normal children that the fasting level did not differ from that of adults. In the cases reported here the fasting value of the blood sugar varied from .067% to .166%, the average being .106%. Three children gave fasting values above .120%. Each of these children was observed for a period of 2 to 3 months in hospital and during that time presented no evidence of a disturbed carbohydrate metabolism. In one child (case III) the high value of .152% was exceptional since on eight other occasions on which the fasting level was estimated it was found to lie between .082% and .103%. Gray[5] in his review of the literature found that 7% of apparently normal individuals have a fasting blood sugar value within .120% and .160%, so that these high fasting values, though unusual, need not be considered pathological. In the work to be subsequently reported it will be noted that/

TABLE III

Case No.	Wt Kgrm	Amount of Glucose grm.	Dur. of Fast hrs.	Fasting level	Percentage Mgs. of Sugar in blood Minutes after Glucose 30	60	90	120	150	180	Sugar in urine
I	27	28	8	.106	.166	.204	.189	.162	–	–	Absent
		100	8	.102	.211	.227	.217	.192	.166	–	"
II	26	27	15	.109	.115	.137	.141	.125	–	–	"
		100	15	.090	.162	.202	.216	.181	.198	–	"
III	22	22	15	.095	.179	.159	.129	.113	–	–	"
		100	8	.152	.256	.341	.321	.264	.241	.196	Abs. at ½hr +at 1 & 3hr
		100	8	.102	.211	.239	.268	.251	–	.211	Absent
IV	28	28	15	.100	.125	.152	.109	.121	–	–	"
		100	15	.100	.179	.179	.191	.129	.131	–	"
V	23	24	8	.119	.204	.196	.158	.111	–	–	"
		100	15	.098	.214	.243	.286	.296	.196	–	"
VI	27	27	8	.113	.137	.147	.141	.109	–	–	"
		100	8	.106	.184	.177	.141	.108	–	–	"
VII	25	25	15	.127	.181	.189	.123	.141	–	–	"
		100	8	.100	.250	.310	.270	.207	.139	–	"
VIII	23	23	15	.141	.152	.196	.172	.129	–	–	"
		100	15	.166	.191	.193	.226	.234	.191	–	"
IX	21	21	8	.110	.174	.193	.168	.094	–	–	"
		121	8	.072	.207	.246	.225	.211	–	–	Abs. at ½& 1hr: +at 1½ hrs.
X	18	18	15	.067	.129	.113	.081	–	–	–	Absent
		88	8	.088	–	.182	.286	.213	–	–	Abs. at ½ & 1hr: + at 1½ hrs
		88	15	.088	.184	.216	.184	.168	–	–	Absent

that the fasting blood sugar value usually falls within the limits of .070% to .120%.

The duration of the starvation did not seem to affect the fasting level, the average value being .106% after an 8 hour fast and .107% after a period of 15 hours without food. This fasting value was not constant for the same individual, but varied somewhat on the different days on which it was estimated. Other observers have noted this same fact and Hale-White and Payne (10) found that the same individual did not give similar types of curves on different occasions.

With one exception, the peak of the curve occurred within the hour after the smaller amount of glucose. The average highest value reached was .173%, the maximum and minimum being .204% and .141% respectively. Generally speaking the results agree with those obtained by MacLean (8) and other workers for the normal adult after a meal of 50 grm. of glucose, except that the curve does not return so quickly to the normal fasting level. This may be due to the relatively larger amount of glucose which the children ingested, or to the longer fasting period, or to a combination of both these factors. It is well known that a second dose of glucose, ingested during a falling blood sugar, produces a very slight (if any) secondary rise. This is usually/

usually explained on the hypothesis that the carbohydrate storage mechanism of the body is already mobilised and able to deal immediately with the second quantity of glucose. Since many of the workers, including MacLean and De.Wesselow[11] have used fasting periods of only 3 to 5 hours it may be that the carbohydrate storage mechanism was, in their cases, not yet "demobilised" and could deal quickly with any glucose ingested, hence the more rapid fall to fasting level recorded by these workers than was found in our series, in which the fasting period was either 8 or 15 hours. It has not, however, been universally accepted that the fasting level should be reached within 2 hours. Fries and Kohn[12] found that only 83% of normal children, receiving less than 2 grm. of glucose per kgrm. of body weight, return to the fasting level within three hours and Hale-White and Payne[10] suggest that a value of .120% is quite within normal limits at the end of two hours. Fries and Kohn[12] also state that the fasting blood sugar, the tolerance and the type of curve registered may vary from day to day.

With the larger amount of glucose somewhat different results were obtained. The maximum value was very much higher, the average being .25% and the maximum and minimum values obtained were .341% and .184% respectively. The

maximum/

maximum value was attained in 50% of the cases at a time later than was the case after the small quantity of glucose. In only one instance (case VI) was a curve, resembling that described by MacLean,[8] noted. MacLean and De Wesselow[11] maintain that in normal individuals the height of the curve cannot be raised above the maximum 18% by large amounts of glucose. though the fall may be delayed. Hale-White and Payne[10] report blood sugar curves rising above .180% with amounts larger than 25 grm. and Foster[13] quotes three cases where the blood sugar reached .214% in 30 mins., .204% in 35 mins., and .201% in 45 mins. after 100 grm. of glucose. Gray[5] found in 300 normal cases a large number in which the blood sugar curve rose to values between .20% and .25% with 100 grm. glucose. In children Fries and Kohn[12] found 20% of their cases rose above .20% with quantities of glucose from 1.5 to 2 grm. per kgrm. of body weight.

From the relationship between the blood sugar level and the glycosuria in this series of cases the renal threshold in childhood would seem to be very high compared to that in the adult. The average value of the renal threshold for glucose in the children in this series seems to lie above .238%. In cases III, IX and X the value can be fairly definitely fixed. In case III it lies within .268% and

.341%/

.341%; in case IX above .246%; and in case X between .216% and .286%. The test with the larger dose, was repeated 4 and 6 days later, in cases III and X. The resulting blood sugar values were not so high as those obtained with the previous test, and no sugar appeared in the urine. Besides enabling a more definite value to be places on the renal threshold in these two cases, these results point to the variability of the tolerance for glucose in the same child on different occasions. The values .296% and .310% in cases V and VII are exceptionally high.

It might be said that the blood sugar did not remain at these high levels sufficiently long for sugar to appear in the urine in amounts possible of detection by ordinary clinical tests. But in cases II and VIII the level of the blood sugar was above .200% for at least half an hour, in case I for one hour, in cases V and VII for two hours and in case IV for three hours. It can be seen, therefore, that this criticism is not valid, the time during which the blood sugar was at a high level being sufficiently long for sugar to have been excreted in easily recognisable amounts.

Glucose has been regarded by the majority of workers as a threshold substance, though Benedict and Osterberg[14] maintain that there is no threshold for glucose and no sudden point at which sugar appears in the urine. MacLean and De Wesselow[11]

and De Wesselow[11] consider the renal threshold as .180%; Olmstead and Gay,[15] and Graham,[16] place it at .190%. Hale-White and Payne[10] suggest that .200% would be a more reasonable value to consider as the normal renal threshold. Mackay,[17] who examined the blood sugar of 44 patients during anaesthesia, found that of 30 subjects who developed glycosuria 37% had maximal blood sugar values of less than .180%. In 14 of the cases in whom glycosuria was not produced 86% had maximal values varying between .180% and .386%. In the case registering the maximal rise of .386% the blood sugar remained above .200% for four hours without any sugar appearing in the urine. Mackay[17] suggests, therefore, that the renal threshold may vary considerably in different individuals and Greenwald, Gross and Samet[18] who also subscribe to this view, think that the threshold may vary in the same individual at different times. Hatlehol[19] also noted this fact. Graham[16] reports a case where a blood sugar of .300% was obtained without any glycosuria. Host[20] found that of 14 normal women, two gave a maximal value of .200% without glycosuria resulting. Widnas[21] also reports normal children with the renal threshold above .230%.

The examples from the literature quoted above show that a higher renal threshold than .180% does exist in the normal adult and that it may be even above .20% but no cases have/

Table IV

Case	Wt Kilos	Amount of Glucose	Fasting level	Percentage (Mgs.) of Blood Sugar. Mins. after Glucose. 30	60	90	120	150	180	210	240	Urine
S.G.	70	210 GRM.	.127	.226	.162	.166	.141	.141	.100	.141	–	Sugar at ½hr only
N.M.	60	180 GRM.	.109	.159	.139	.136	.125	.128	.094	–	.077	No sugar
M.G.	43	130 GRM.	.127	.191	.190	.184	.141	.141	.135	–	–	No sugar
O.M.	60	160?* GRM.	.109	.157	.166	.141	.141	.134	.134	–	–	No sugar

* Slight sickness about 10 minutes after ingestion.

have been described where after ingesting glucose such typically diabetic curves have been obtained. In the present series of cases, with a large quantity of glucose, 80% showed a high renal threshold and diabetic type of curve. It must be remembered, however, that blood sugar curves following amounts of glucose relatively as large as those given to the subjects in this present series (i.e. roughly 4 grm. per kgrm. of body weight) have rarely been estimated in the adult.

It is interesting to note here that an attempt to estimate the effect of such large amounts of glucose on the blood sugar of four adults was made, but the most that they were able to retain without sickness was 3 grm. per kgrm. of body weight. The details are found in Table IV.

It can be seen that in only one, S.G., was the hyperglycaemia above .200% and here the renal threshold for glucose was passed and sugar appeared in the first ½ hour specimen of urine but not in any of the other specimens. The large quantities of glucose given, though relatively not so large as those ingested by the children, do not seem to raise in the adult, the height of the blood sugar curve so readily as in the child. We consider that the tolerance for glucose in a child, as revealed by the blood sugar curve, is not so high as that of the adult and that probably the renal threshold is at/

at a somewhat higher level.

The renal threshold in diabetics varies but is usually well above .200%, and two cases of diabetes mellitus observed in the Royal Hospital for Sick Children show renal threshold values above .253% and .282%. John (49) reports a case of diabetes mellitus with a blood sugar level of .390% and no sugar in the urine. It has generally been assumed that this has been raised above the normal in the diabetic. It might be suggested, therefore, in view of the results obtained in the above investigation that in a certain number of cases of diabetes mellitus the level of the renal threshold has not been raised but is at its normal level.

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SECTION III

In this section are detailed and compared the results of the blood sugar curves after

I. The ingestion of 1 grm. of glucose per kgrm. of body weight.

II. The ingestion of 100 grm. of glucose.

III. The subcutaneous injection of Insulin.

IV. The subcutaneous injection of Pituitrin.

V. The effect of Pituitrin feeding on Carbohydrate Tolerance.

VI. The subcutaneous injection of ~~Insulin~~ IN sulin, and

VII. The Laevulose Test,

under the condition of

A. A normal diet, and
B. A ketogenic diet.

By the term "ketogenic" diet is meant a high fat diet which consisted of varying proportions of fat bacon, butter, cream, curds, fish, cheese and 5% vegetable. The amounts varied slightly according to the caloric requirements of the child. Tea with saccharine and water were given to drink. The Ketogenic-Anti-Ketogenic ratio of this diet was approximately 3.5 to 1, as calculated from Shaffer's[1] data, and in every case acetonuria or ketosis developed within 36 to 48 hours and continued throughout the duration of the experiment. This high fat diet was similar to that given by Brown and Graham[2] in their work on "Ketonaemia and Ketonuria in Childhood" and/

and they found in every case an increase in the blood acetone accompanied by a fall in the alkaline reserve. They assumed that "in the absence of a definitely increased CO_2 output by over ventilation a reduction in the alkaline reserve must be taken as an indication of a reduction in the serum pH, and consequent acidosis." Acidosis occurred in their cases without giving any clinical manifestations of its presence and the same holds good for the series reported here. No blood analyses demonstrating the existence of an acidosis were carried out in this work, but it was taken for granted on account of Brown and Graham's[2] findings that such a condition did exist.

The acetone in the urine was estimated qualitatively only by means of Rothera's Test. A 24 hour specimen of the urine was examined daily during the period of the experiment and it was found that the acetone which was +++ in quantity during the first seven days or so, decreased to a + amount after this with occasional daily variations to a ++ or a ±, the cause of which was not manifest. An attempt was made in all the experiments of this section to collect ½ hourly specimens of urine during a blood sugar test, the urine being collected immediately after the specimen of blood had been withdrawn. Occasionally, however, specimens of urine were not obtained with such regularity as this.

Table V.

* 0 = Normal mixed Hospital Diet.
F = Special high fat diet, Ketogenic Diet.

Case	Wt Kgrm	Amount of Glucose grm.	Dur. of Fast hrs	Percentage Mgs. of Sugar in Blood — Fasting level	Minutes after Glucose: 30	60	90	120	150	Diet *	Sugar and Acetone in Urine
I.	28	28	8	.106	.166	.204	.189	.162	–	0	Nil
			15	.072	.072	.168	.201	.193	–	F	No.Sug. Acet.+
II.	27	27	15	.109	.115	.137	.141	.125	–	0	Nil
			15	.092	.082	.134	.211	.221	.231	F	No Sug. Acet.+
III.	22	22	15	.095	.179	.159	.129	.113	–	0	Nil
			8	.072	.164	.224	.234	.179	.177	F	No Sug. Acet +
IV.	28	28	15	.100	.125	.152	.109	.121	–	0	Nil
			8	.062	.075	.148	.159	.162	–	F	No Sug. Acet +
V.	24	24	8	.119	.204	.196	.158	.111	–	0	Nil
			8	.077	.134	.182	.221	.187	–	F	No Sug. Acet.+
VI.	27	27	8	.113	.137	.147	.141	.109	–	0	Nil
			15	.072	.103	.159	.141	.162	–	F	No Sug. Acet.Tr.
VII.	25	25	15	.127	.181	.189	.123	.141	–	0	Nil
			15	.100	.162	.218	.206	.177	–	F	No Sug. Acet.+
Composite Curve				.109	.158	.169	.141	.126	–	0	–
				.078	.113	.176	.196	.188	–	F	–

Part I.

The Blood Sugar Curve on

A. Normal Diet, and
B. "Ketogenic" Diet.

The subjects of all the experiments in this and the remainder of the work in Section III were the first seven cases described in Section II, and as the results of the blood sugar curves on normal diet with 1 grm. of glucose per kgrm. of body weight and with 100 grm. of glucose have been dealt with fully in the latter section, neither of these results will be considered in detail here.

Table V gives the details of the blood sugar curves and Chart I shows graphically one typical result and the composite curve of all the tests. Chart Ia contains the graphic results of the other cases.

TABLE V

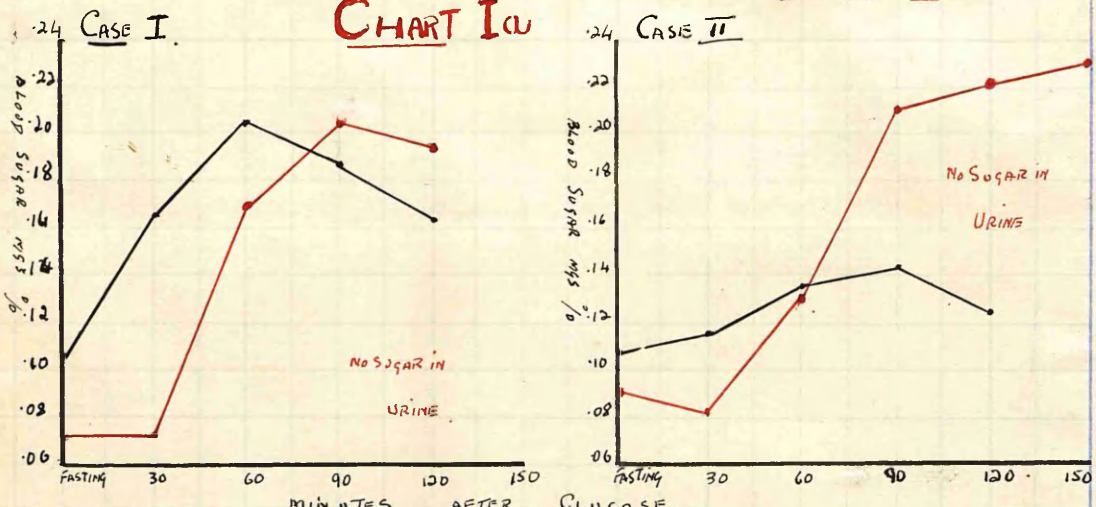
CHART I

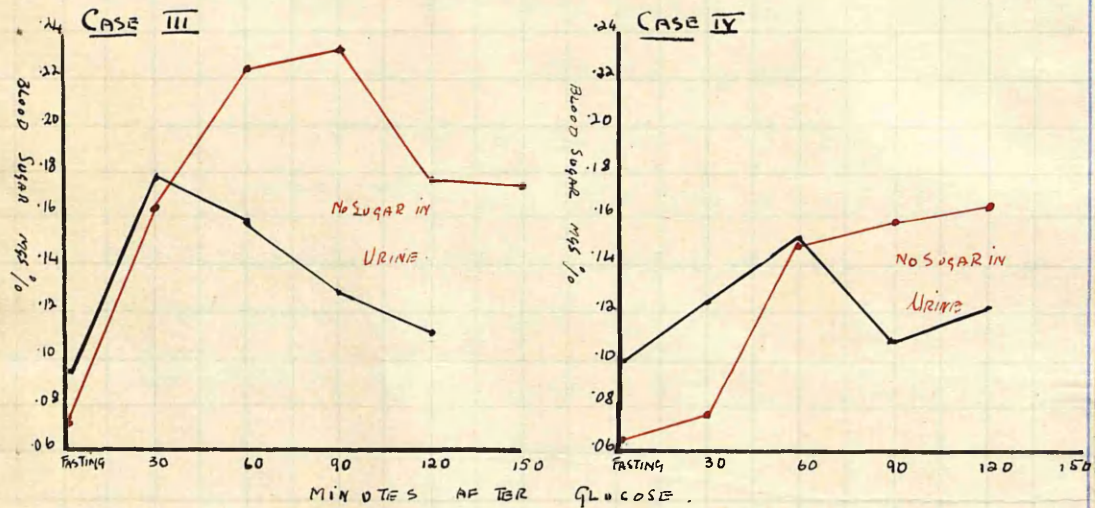
BLOOD SUGAR CURVES AFTER 1gRm of GLUCOSE.

ON ORDINARY DIET = ●——●

KETOGENIC DIET = ●——●

CHART Icu





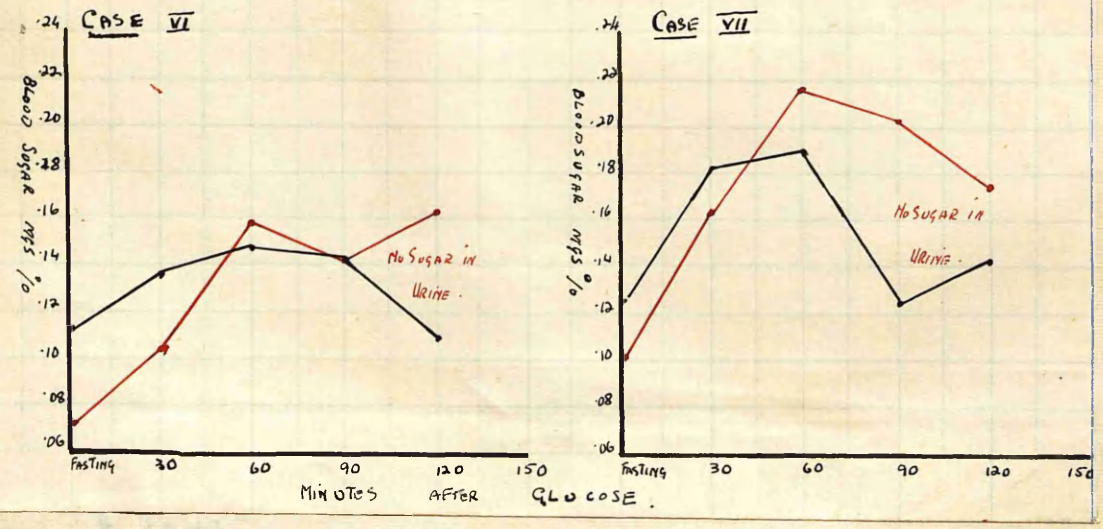
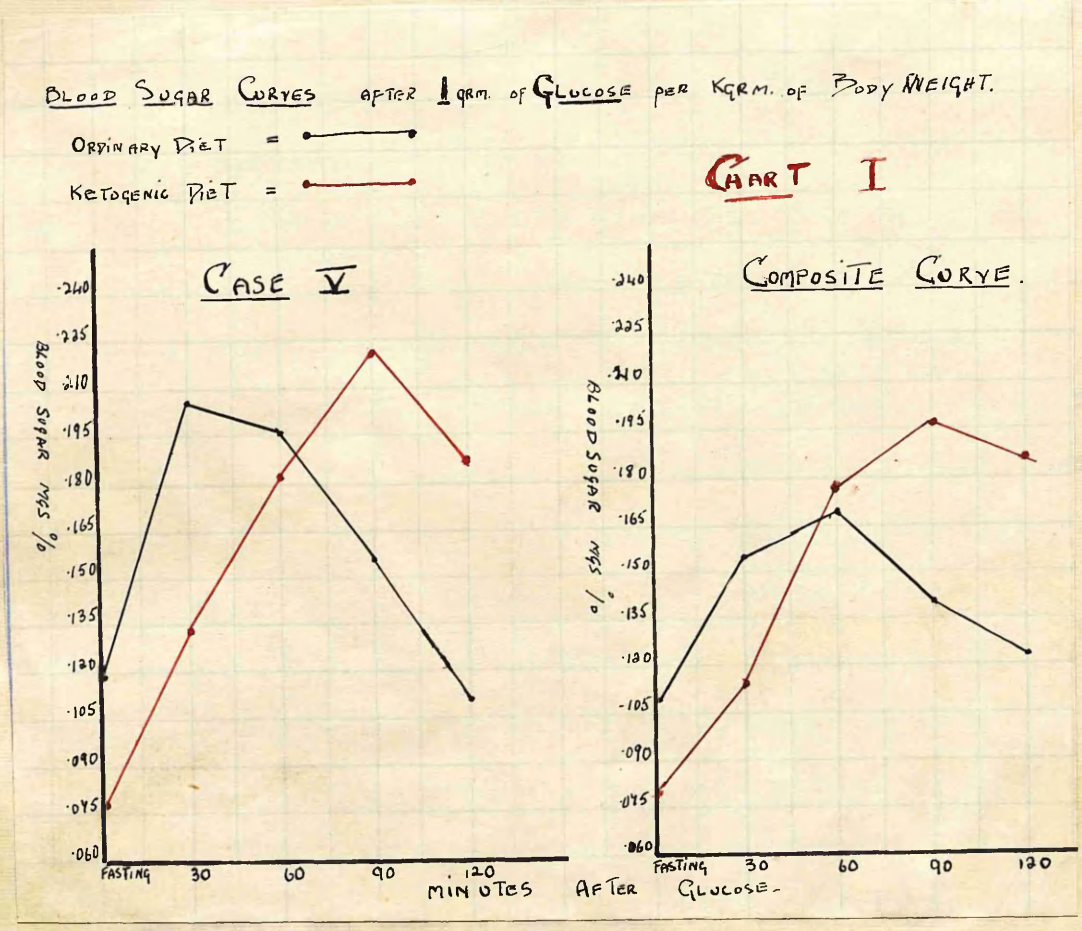


Chart I.



The results reveal that the change in diet has produced a reduction in the tolerance for glucose, the curves resembling those obtained in a mild case of diabetes mellitus. The fasting blood sugar is at a lower level, in every case, than on normal diet. These experiments were/

were carried out after 10-22 days on a "ketogenic" diet and the values are not quite so low as those obtained by the 3rd to the 4th day. Although the degree of hyperglycaemia attained is much greater on the high fat diet, the curve shows a much slower rise, the rise in blood sugar at the ½ hour being less than on a normal diet. Indeed in three of the cases, I, II and IV the value is practically the same as that found at fasting. In every case except case I, the peak of the curve is higher than on normal diet but the most striking point is the delay in the return of the blood sugar to fasting level. A more marked lowering of tolerance as regarded by the height and configuration of the blood sugar is shown in cases II and III who were for a longer period on the ketogenic diet than any of the other children.

In cases II, III, V and VII, the blood sugar rose to values between .218% and .234% and no sugar appeared in the urine. This conforms to the renal threshold level for glucose described in Section II. Case VI did not show a very marked change in the hyperglycaemia attained on a high fat diet but the value at the end of two hours is still elevated much above the fasting level.

Acetone was a + quantity in the urine at the beginning of each experiment, except in case VI where only a trace was/

was registered. That the acetone output varied slightly was noted on a daily examination of a 24 hour specimen of urine in all of the cases and by the following day the acetone was again a + quantity in this case. In case IV the acetone had gone from the urine two hours after the ingestion of the glucose and in case V it had been reduced to a ± quantity.

Part II.

The Blood Sugar Curve after 100 grm. of glucose on

A. Normal Diet, and
B. Ketogenic Diet.

The details are found in Table VI and two representative cases in Chart II are shown graphically.

TABLE VI.

CHART II

Table VI.

Case	Wt Kgrm	Amount of Glucose grm.	Dur. of Fast hrs.	Percentage of Sugar in Blood: Fasting level	Minutes after Glucose: 30	60	90	120	150	180	Diet *	Sugar and Acetone in Urine.
I.	28	100	8	.102	–	.211	.227	.217	.193	.166	O	No.Sug. Acet –
			8	.092	.196	.326	–	.331	.316	.302	F	Sug.++ 1h Acet +
II	27	100	15	.090	.162	.203	.216	.181	.198	–	O	Nil
			8	.081	.191	.321	.351	.393	.421	–	F	Sug.++ 1h Acet.+
III †	22	100	8	.102	.211	.231	.268	.251	.211	–	O	NNil
			8	.084	.226	.346	.376	.409	.407	–	F	No Sug. ¾hr) Acet Sug.++ 2¾hr) +
IV	28	100	15	.100	.179	.179	.191	.129	.131	–	O	Nil
			15	.089	.214	.264	.277	.229	.211	–	F	Sug.+ at 1½ hrs. Acet +
V	24	100	15	.098	.214	.243	.286	.296	.196	–	O	Nil
			8	.081	.189	.331	.361	.321	.326	–	F	F.Tr.Su.1 hr. Sug.++ 1½ hrs Acet +
VI	27	100	8	.106	.184	.177	.141	.108	–	–	O	Nil
		100	15	.100	.193	.191	.195	.179	.164	–	F	No Sug. Acet +
		130	15	.069	.234	.266	.302	.291	.279	.249	F	Tr.sug.½hr Acet+
VII	25	130	15	.100	.250	.310	.270	.207	.139	–	O	Nil
			15	.084	.152	.271	.311	.292	.278	–	F	Sug.+ at 1hr Acet ++

* O = Ordinary mixed hospital diet. † See Table III also and Text
F = Special high fat diet,""Ketogenic" diet

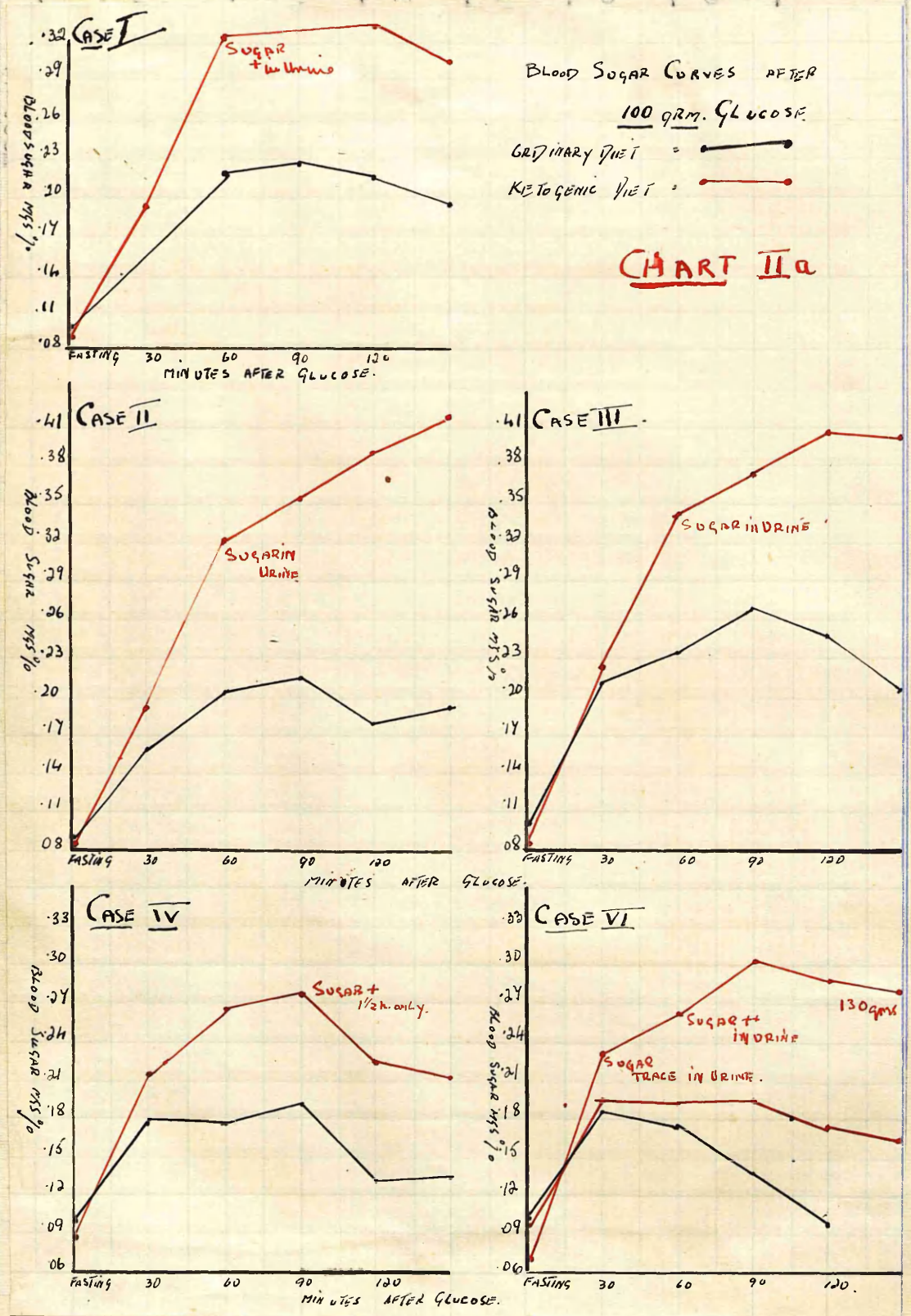
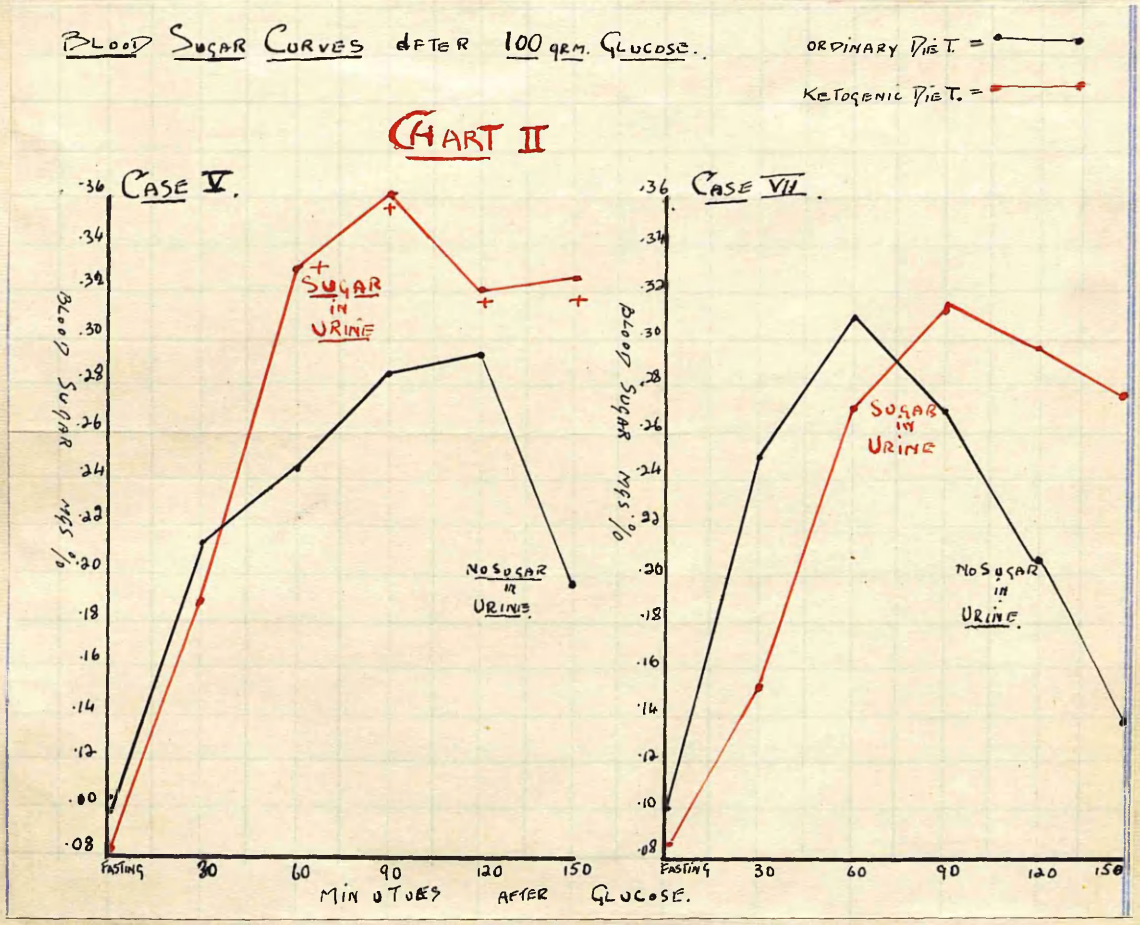

CASE I
SUGAR + in Urine
BLOOD SUGAR MGS %
.32
.29
.26
.23
.20
.17
.14
.11
.08
FASTING 30 60 90 120
MINUTES AFTER GLUCOSE.
BLOOD SUGAR MGS %
CASE II
SUGAR IN URINE
.41
.38
.35
.32
.29
.26
.23
.20
.17
.14
.11
.08
FASTING 30 60 90 120
MINUTES AFTER GLUCOSE.
CASE III
SUGAR IN URINE
BLOOD SUGAR MGS %
.41
.38
.35
.32
.29
.26
.23
.20
.17
.14
.11
.08
FASTING 30 60 90 120
CASE IV
SUGAR + 1½ h. only.
BLOOD SUGAR MGS %
.33
.30
.27
.24
.21
.18
.16
.12
.09
.06
FASTING 30 60 90 120
MINUTES AFTER GLUCOSE.
CASE VI
130 gms
SUGAR + IN URINE
SUGAR TRACE IN URINE.
BLOOD SUGAR MGS %
.33
.30
.27
.24
.21
.18
.15
.12
.09
.06
FASTING 30 60 90 120.
BLOOD SUGAR CURVES AFTER 100 GRM. GLUCOSE
ORDINARY DIET =
KETOGENIC DIET =
CHART IIa

Chart II.



As was to be expected, the results are similar in kind though different in degree, to those reported above. The tolerance for 100 grm. of glucose was much reduced and in every case, except VI, the renal threshold was passed and glycosuria/

glycosuria resulted. The tolerance in case VI was broken by 130 grm. of glucose but he was able, on a normal diet, to take 270 grm. without sugar appearing in the urine. This child's tolerance for glucose would seem to exceed that of the other cases and his threshold level appears to be nearer the value .200%. The results in Section II indicate that the renal threshold in childhood might be higher than in the adult and if this were so it might be that this child was approaching nearer to the adult type of metabolism than the others. He was not, however, the oldest child in the group, being 11 years only, whereas two other children were aged 12 years. Fries and Kohn (12) found that in a series of normal children those with the poorest tolerance were aged 7 to 10 years and that the tolerance was better under 7 and over 10 years of age. We have not been able to show in our results any such variation of tolerance due to the age of the child.

As can be seen from Chart II the hyperglycaemia attained after 100 grm. of glucose on a carbohydrate free diet is very marked and very prolonged. Cases II and III reached the very high blood sugar values of .421% and .409% at the end of the 2½ hours and showed no tendency to return to fasting level at this time. No sugar appeared in the urine at/

at the ½ hour period in any of the cases but was abundantly present at the hour period, except in case IV. In her case glycosuria occurred at the 1½ hour period only and this has enabled the threshold level to be placed definitely above the value .264%. In case VII there would seem to have been a lowering of this renal threshold since sugar was present in the urine on the ketogenic diet period when the blood sugar level was .271% though on a normal diet no sugar was excreted with a hyperglycaemia of .310%.

It was noted that after this large quantity of glucose there was a mild duiresis and in case V this was particularly marked, a urine of specific gravity 1000 being passed during the test on normal diet. On ketogenic diet the Specific Gravity of one specimen of urine, loaded with sugar, was 1003. This is somewhat contradictory to the usual teaching that urine containing sugar has a high Specific Gravity. It has been remarked, however, that in two cases of diabetes mellitus observed in the Royal Hospital for Sick Children urine containing sugar and yet having a Specific Gravity of 1002 has been passed.

The quickness with which glucose can clear acetone from the urine is very striking. In cases I and VII only a faint trace remained 2½ hours after the ingestion of 100 grm. of glucose. In cases II, IV, V and VI no acetone was present in/

in the urine at the end of the test period and in case IV it had gone completely by the end of 1½ hours. Glucose is much more effective in this antiketogenic quality than laevulose, as will be demonstrated further on in this thesis, and Goldblatt (22) found that in starvation ketosis, glucose removed acetone from urine in 1 hour and it did not return for 3½ hours. Laevulose, on the other hand, overcame ketosis in 1½ hours only and preserved the balance but for one hour.

It is therefore, very definitely demonstrated that a high fat diet lowers the tolerance for glucose and raises the blood sugar to a phenomenally high value. The fasting blood sugar is lowered below the normal, the curve does not rise so quickly as on ordinary diet, and there is marked prolongation of the time required for the blood sugar to return to fasting level.

Clinical Considerations.

Before going on to discuss the above results it seems worth while to draw attention to a point which may be useful in the clinical diagnosis of the different type of glycosuria and to differentiate between those which require treatment and those which do not.

In the frank case of diabetes mellitus which comes to the physician with symptoms of thirst, polyuria, weakness and emaciation/

emaciation, of whatever degree, there is little likelihood of a mistaken diagnosis being made. But in the case where glycosuria is discovered accidentally (e.g. in a life insurance examination) and the individual put on a low carbohydrate diet and the sugar tolerance then tested a mistaken diagnosis may be made. If the person is not a diabetic the low carbohydrate diet may produce a curve indicative of mild diabetes, as shown above, and may be wrongly labelled as suffering from diabetes mellitus. On the other hand the opposite may occur. This point can be illustrated by the case of a child in the Diabetic Clinic of the Royal Hospital for Sick Children.

This child, J.L., had had an attack of whooping cough and during his convalescence he was noticed to have some increased thirst. The urine was examined and found to contain sugar. He was put on a low carbohydrate diet, the sugar disappeared and he seemed quite well. On admission to hospital for an investigation into his case a five hour fasting blood sugar was only .106%. After a fifteen hour fast and 16 grm. of glucose the blood sugar curve was as follows:

F.B.S.	½hr.	1hr.	1½hr.	2hr.
.075%	.115%	.119%	.164%	.154%

A/

A two hour specimen of urine contained Acetone + but no sugar. This blood sugar is normal apart from the delay in return to fasting level. He was given ordinary diet for two days, no sugar appeared in the urine and a five hour fasting blood sugar was .116%, still within normal limits. After six days on this diet the following was the blood sugar curve recorded after a fifteen hour fast and 16 grm. of glucose.

F.B.S.	½hr.	1hr.	1½hr.	2hr.
.152%	.184%	.199%	.259%	.279%

There was a trace of sugar in urine at 2hr. period only and no acetone.

This is a frankly diabetic curve. If he had been normal his tolerance for glucose, on the ordinary diet, should have been increased, not reduced.

The following rule may be formulated for doubtful cases of glycosuria. The blood sugar curve should be estimated on

(a) a normal diet, and
(b) a low carbohydrate diet.

If the individual is normal as regards his glucose metabolism, he will show a better tolerance for glucose on an ordinary diet than on the low carbohydrate diet. If he is a diabetic the findings will be reversed.

This rule is useful also in helping to decide the difficult/

difficult point whether a person with renal glycosuria should be treated as a potential diabetic. A patient, H.F., who had been on a low carbohydrate diet after the accidental discovery of glycosuria, had his sugar tolerance tested. The blood sugar after 50 grm. of glucose was considered normal.

F.B.S.	½hr.	1hr.	1½hr.	2hr.
.100%	.210%	.160%	.110%	.100%

He was given ordinary diet for eight weeks and his sugar tolerance estimated again. The result revealed that there was a lowering of the tolerance for glucose.

F.B.S.	½hr.	1hr.	1½hr.	2hr.
.098%	.179%	.268%	.170%	.141%

He was advised to keep to the low carbohydrate diet for though the curve cannot be called diabetic in that it is nearly at fasting level in two hours yet it revealed a slight weakness of the carbohydrate disposing mechanism on ordinary diet.

It is important, therefore, before performing this customary sugar tolerance test used in clinical diagnosis that the previous period of diet should be carefully controlled. This point has been noted before by several other workers, but we do not think it has been sufficiently stressed in text books dealing with the diagnosis and treatment of diabetes mellitus and glycosuria.

Discussion/

Discussion.

Other workers have observed this lowering of the tolerance for glucose produced by the administration of a carbohydrate free diet. Kageura[23] noted this fact in dogs fed on a high fat diet and Southwood[24] obtained a much enhanced hyperglycaemic reaction in men who had been given a carbohydrate free diet for 36 hours previous to the test. Staub[25] and Greenwald, Gross and Samet[18] reported similar findings. Kohn, Fries and Felshin[26] obtained "diabetic curves" in children, also suffering from chorea, who were receiving a diet with the Fatty Acid:Glucose Ratio of 2.5 to 1 or 3:1.

This reduction of sugar tolerance arises not only with a carbohydrate free diet but also occurs if individuals are starved for any period of time. Severinghaus[27] found, after fasting for at least 48 hours, that the ingestion of glucose resulted in a blood sugar reaction, previously normal, resembling that of a mild diabetic and Du Vigneaud and Karr[28] and Lennox,[29] among others reported similar results.

Besides the lowering of tolerance for glucose, revealed by the degree of hyperglycaemia attained, the fasting blood sugar of subjects receiving a carbohydrate free diet, or no diet at all, is always lower than the normal fasting level. This reduction of the fasting sugar content of the blood is more/

more marked during the first few days of a "ketogenic" diet or a fast and in this series of cases the average fasting blood sugar was .071% for the first three to four days, whereas by the 22nd to 26th day the average had risen to .083%. Salomonsen (30) found on a high fat diet that the fasting blood sugar was lowest on the second day, remained low till the fourth day and by the sixth day had returned to a low normal level. Ross and Joseph (31) and Talbot, Shaw and Moriarty (32) found that the lowest value occurred during a period of starvation on the fifth and sixth days and then tended to return to normal fasting level.

That the blood sugar, however, once it has recovered the normal level does not, in every case, remain there is shown by the fasting blood sugar values obtained in case VI during the period on "ketogenic" diet.

No.of Days on Ketogenic Diet	3	5	10	12	15	17	19	23
Fasting Blood Sugar	.063	.081	.071	.085	.089	.100	.069	.067

It is remarkable that in the series of cases here described none of the children showed any clinical symptoms of hyperglycaemia or acidosis. The children did not suffer from sickness, lassitude, disturbance of respiration nor restlessness and seemed in no way disturbed in their general health/

health. This fact has been commented upon by Brown and Graham (2) and Salomonsen (30) in his series of 12 normal children noted vomiting in four cases only, though in none were the symptoms at all comparable to that seen in cyclical vomiting. A similar freedom from the manifestations of any severe clinical symptoms in the acidosis produced from starvation is reported by Weymuller and Schloss (33), and Talbot, Shaw and Moriarty (32). Three of the subjects in the investigation by Weymuller and Schloss (33) became drowsy with acetone odour in the breath after a 48 hour fast while on a ketogenic diet. Talbot, Shaw and Moriarty (32) had to give glucose to remove the symptoms of acidosis on the third day of a fast in only one of their cases. This child had on that day a blood sugar of only .038%.

This point of hyperglycaemic values in the blood sugar during a short fast of eight to fifteen hours on a high fat diet or during the first six to seven days of a fast is of interest in that there are no manifest clinical symptoms of hyperglycaemia. One child, M.McI, during the course of another investigation not recorded in this thesis, had a fasting blood sugar of .040% on the seventh and seventeenth days of a ketogenic diet and seemed quite undisturbed. Weymuller and Schloss (33) report two children with fasting blood sugar values of .042% and .035% without any symptoms of hypoglycaemia/

hypoglycaemia. They consider that some other factor besides the low sugar content of the blood is concerned in the production of the symptoms described as occurring with hypoglycaemia. This question will be further discussed in the section dealing with the hypoglycaemia produced by insulin.

The low fasting level of the blood sugar may be accounted for by supposing the liver to have become depleted of its glycogen to a greater or lesser degree and further evidence to show that this inhibition of glycogenic function does exist under the changed conditions of a high fat diet will be submitted later. As the length of fast, or the period of administration of the diet, increases, the blood sugar tends to rise again. This may be due to a compensatory formation of glucose from protein or fat or to a lessening of the severity of the ketosis or acidosis which may be the cause of the inhibition of the glycogenic function of the liver. Brown and Graham (2) demonstrated that the ketosis was less marked after the eighth day.

A non-gaseous acidosis accompanied by a ketosis is produced by a high fat diet and a fast over a period of several days, produces similar changes in the biochemistry of the blood. In both of these conditions there occurs a failure in the metabolism of carbohydrate. What factor is it that produces the disturbance of metabolism? Is it the lack of carbohydrate/

carbohydrate in the diet, the disturbance in the acid-base equilibrium or the ketosis, which is responsible for the change?

It has always been recognised that the liver plays an important part in the metabolism of carbohydrate and an attempt has been made in the following pages to show that the glycogenic function of the liver has been interfered with. Evidence will be brought forward to show that it is probably the ketosis which is the causal factor in this change in the efficiency of the liver and these findings will be discussed along with the various theories previously propounded regarding the mechanism of production of the lowered tolerance for glucose accompanying a "ketogenic" diet.

Part III

The Subcutaneous Injection of Adrenalin.

The next results to be considered are those obtained on

A. a Normal Diet, and
B. a "Ketogenic" Diet,

after the injection of adrenaline chloride (1/1000 solution - B.W. 60). The smallest quantity of this solution of adrenalin which caused a definite rise in the blood sugar in children of different ages was worked out at the Royal Hospital for Sick Children by Dr. I. Mitchell. It was found that children/

Table VII.

Case	Age Yrs	Minims of Adren. Subcut	Diet *	Percentage (Mgs) of Blood Sugar: Fasting level	Minutes after Adrenaline: 10	30	60	90	120	150	Rise in Blood sugar in Mgs.	Urine.
I.	12	6	O	.104	.104	.117	.132	.132	.100	–	28	Nil +
			F	.075	.075	.085	.100	.090	.072	–	25	No sug. Acet.++
II.	10	5	O	.089	.100	.131	.122	.117	.089	–	42	Nil
			F	.067	.077	.077	.067	.072	.100	.072	33	NoSug.Acet.+++
III.	10	5	O	.084	.134	.107	.117	.105	.111	–	50	Nil
			F	.077	.083	.077	.077	.117	.058	–	40	No Sug.Acet.++
IV.	11	6	O	.100	.116	.129	.132	.125	.116	–	32	Nil
			F	.074	.043	.056	.060	.072	.081	–	–	Acet. ++++
			F	.084	.097	.117	.131	.104	–	–	47	No Sug.Acet.+
V.	12	6	O	.113	.123	.172	.191	.139	.125	–	78	Nil
			F	.079	.102	.119	.129	.100	.094	–	50	No Sug.Acet.+++
VI.	11	6	O	.104	.129	.117	.117	.134	.122	–	30	Nil
			F	.063	.067	.081	.089	.072	.089	–	26	No Sug.Acet.++
VII.	9	5	O	.094	.125	.123	.134	.117	.104	–	40	Nil
			F	.063	.064	.075	.084	.072	.064	–	21	No Sug.Acet +++
Composite Curves			O	.098	.119	.128	.135	.124	.109	–	37	Nil
			F	.072	.081	.090	.097	.089	.079	–	25	– Acetone.

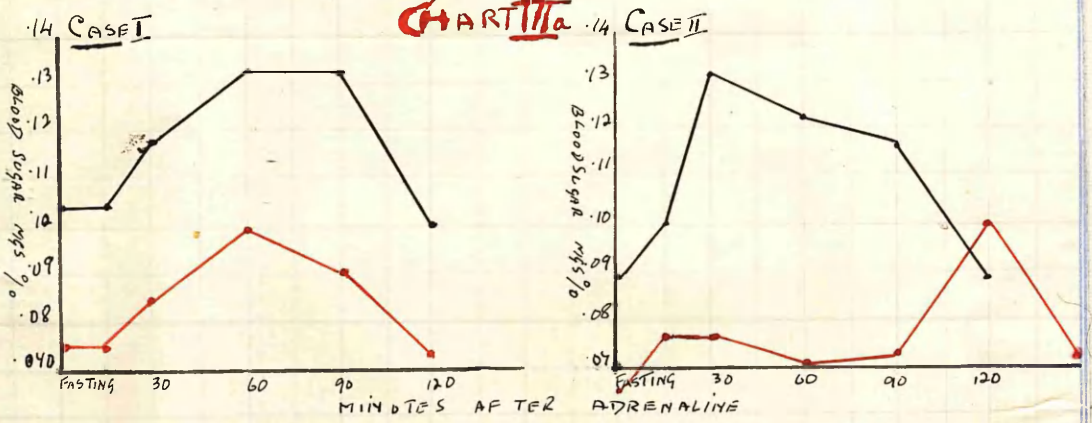
O = ordinary mixed hospital diet F = "ketogenic" diet.

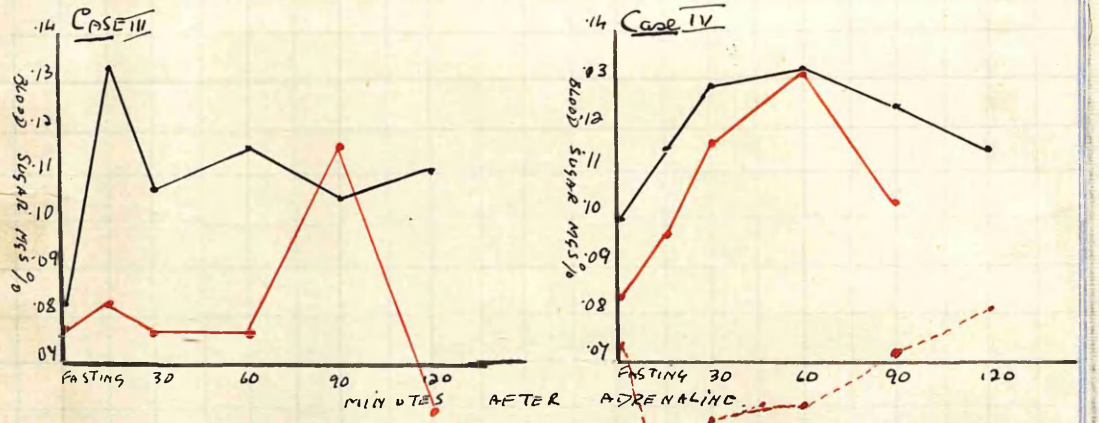
BLOOD SUGAR CURVES AFTER ADRENALINE.

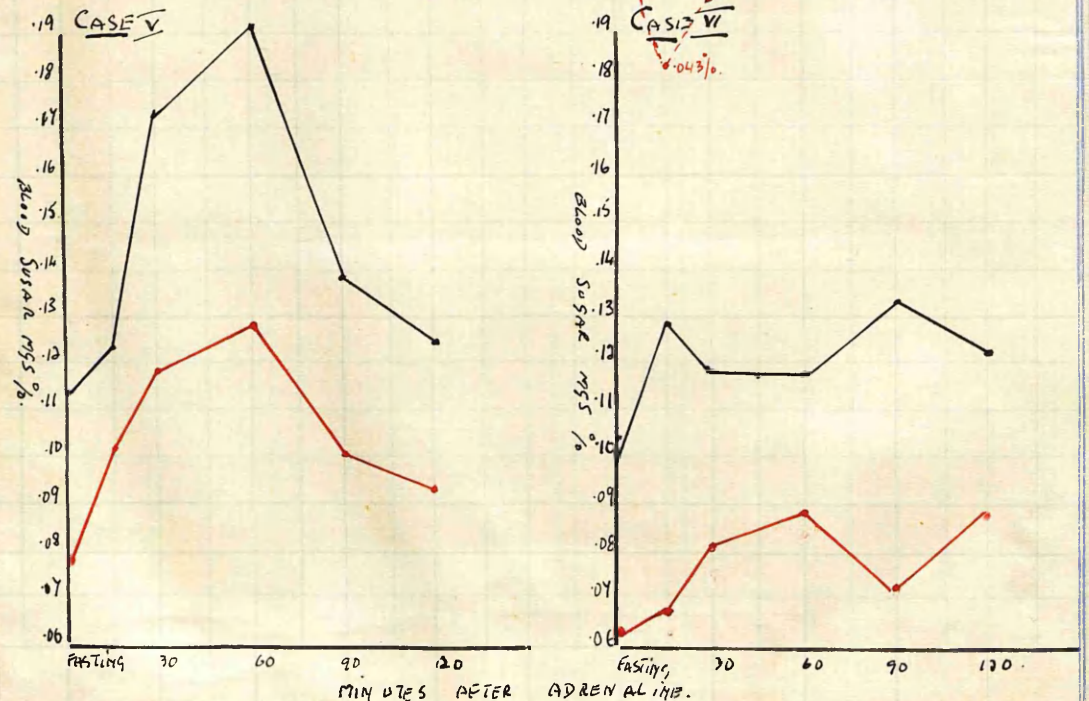
KETOGENIC DIET = ——

ORDINARY DIET = ——

CHART IIIa







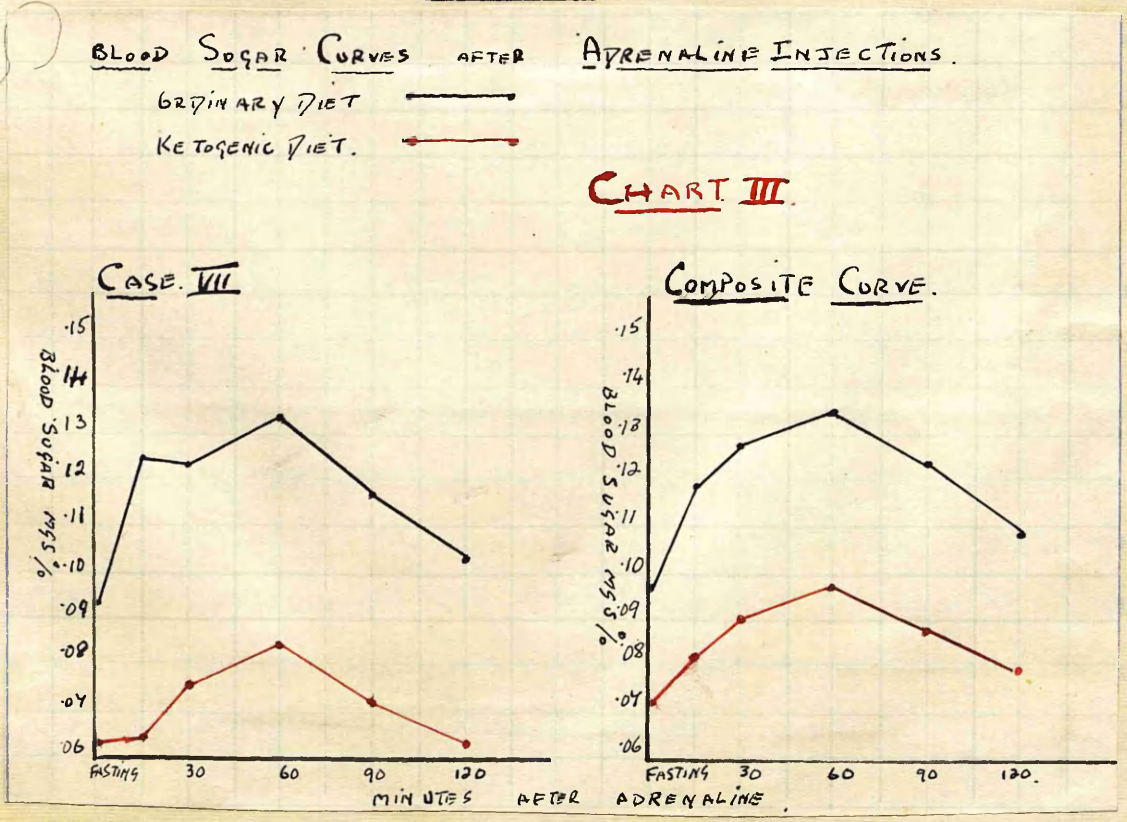
children of different ages reacted to different amounts of adrenalin in the following way.

Amount of Adrenalin required to cause a definite rise in blood sugar.

At 1 year of age	1 minim	of adrenalin
" 2 years" "	2 "	" "
" 3,4,5,6,7 years	3 "	" "
" 8 years of age	4 "	" "
" 9,10,11 years	5 "	" "
" 12 years of age	6 "	" "

In the work detailed below the amounts of adrenalin used were based on this standard. The injections were subcutaneous and the blood for the sugar estimations was taken at fasting and at 10, 30, 60, 90 and 120 minutes after the injection.

Chart III.



Under ordinary dietary conditions it is seen from Table VII that all seven cases showed the normal response which one expects after the injection of adrenalin where there are sufficient reserves of glycogen in the body. In six of the cases a definite rise in the level of the blood sugar had occurred in ten minutes but the maximal rise in four of the cases did not appear till one hour after the injection; in the three remaining cases it occurred at 10, 30 and 90 minutes respectively. In all the cases the fasting level had been reached, or nearly so, by the end of two hours.

It should be noted that in case V the hyperglycaemia resulting from the injection of adrenaline was very marked. This result will be considered along with that of the insulin injection details of which will be found below.

In none of the cases did adrenalin produce glycosuria and there were no symptoms noted nor complained of after the injection.

The results obtained when the children were receiving "ketogenic" diet are not so constant but, on the whole it can be concluded that the response, by a release of glycogen from the tissues into the blood stream is not so good as that which occurs while the subjects are receiving a diet containing a sufficient quantity of carbohydrate.

The/

The difference obtained on the two diets is not very marked if the rise in mgs. from fasting level to maximum value is considered, but when the curves are graphed and the areas compared it is seen that there is a very definite difference, the greater output of glycogen occurring on the normal diet. Cases II, III, V and VII show a very definite decrease in the response to adrenaline while on a high fat diet. The rise in cases II and III is very delayed, occurring at two hours and 1½ hours respectively and is very transitory, suggesting that the readily mobilised glycogen of the liver was much reduced. Case V again reacted well to the adrenaline, though much less so than on ordinary diet. In case I there is only a slight difference but the balance is on the side of the normal diet. This is also the case with VI though the response made to adrenaline, on either diet, is not marked. Case IV is the only one where the difference in mgs. of blood sugar shows a higher rise on the ketogenic diet though again the rise is not so sustained and the area the curves represent is greater under normal dietary conditions.

It is worthy of note that the first curve obtained in this case (case IV) while on a ketogenic diet showed a response which one would expect to obtain after an injection of/

of insulin. The child vomited and did not feel well during the test though this was not known till afterwards. A similar reversal of results was obtained, on normal diet, after injecting insulin. The reason for such behaviour is obscure as on the injections of adrenaline and insulin (see Table VIII) being repeated normal responses were obtained.

Blottner and Fitz[34] have demonstrated that "adrenalised" blood does not contain insulin so it is unlikely that the adrenalin should cause a secretion of insulin thereby producing hypoglycaemia; or, vice versa, that an injection of insulin should cause a release of adrenaline. But Macleod[35] has suggested that the recovery of the blood sugar following insulin is associated with a hypersecretion of adrenaline and it may be that in this case this balanced reaction, if it does exist, is over sensitive and responds too soon, so that the normal reaction is overshadowed by the compensatory one.

Discussion.

It is generally accepted that the hyperglycaemia produced by the injection of adrenalin is due to the release of liver glycogen and that if there is no liver glycogen available no hyperglycaemia occurs. Proof that the liver

is/

is involved in this hyperglycaemia has been given by Mann and Magath (36) who showed that when the liver had been removed injections of adrenaline had no effect and do not prevent the hypoglycaemia which is the result of hepatectomy. Collins, Shelling and Byron (37) also showed that after the arterial exclusion of the liver adrenaline fails to cause an increase in the blood sugar during the resultant hypoglycaemia.

Cori and Cori (38) have suggested that the mobilisation of liver glycogen alone is an inadequate explanation of the hyperglycaemia and that in addition to this adrenalin inhibits the utilisation of the sugar in the peripheral tissues of the intact animal. Du Vigneaud and Karr (28) found that with glucose and adrenaline injected together the resultant curve was higher than the sum of the curves of these two substances when given alone. They therefore suggest that adrenalin prevented the storage of sugar. If this were so one would expect that the blood sugar values would rise even if there were an absence of glycogen in the liver since the adrenaline would prevent the tissues from removing more sugar from the blood and allow whatever glycolysis was proceeding to accumulate in the blood stream. This might account for the rise in blood sugar on a ketogenic diet obtained in a few of the above cases even though there

was depletion of liver glycogen. It is unlikely, however, that the liver was quite devoid of glycogen and that those subjects which showed hyperglycaemia after adrenalin while on a high fat diet still possessed some glycogen in the liver depots.

That the lessened hyperglycaemia is not due to adrenalin stimulating insulin more thoroughly under the condition of a ketosis has been shown by Blottner and Fitz (34) who could not demonstrate insulin in "adrenalised" blood though it was present in "pituitrinised" blood.

Burn and Marks (39) have shown that an increased response to an injection of adrenaline is obtained after feeding rabbits on thyroid for eight days. If however the thyroid feeding is continued for eighteen days a much lower response to adrenalin is obtained. On estimations of the liver glycogen being made it is found that after eight days there is very little decrease in the glycogen but that after eighteen days the reserves of liver glycogen are much depleted.

McDaŋell and Underhill (40) found that rabbits fed on an acid forming diet gave a lower response to adrenalin than rabbits on a base forming diet or a mixed diet. The base forming diet, consisting of carrots, is a much heavier carbohydrate diet than the acid forming diet, and lays down a heavy deposit of glycogen in the liver of the rabbit. The difference/

difference in these two results might be explained on the variation of available glycogen and not on the change in H ion. concentration.

It may be that adrenaline is not such an effective mobiliser of glycogen under the disturbance of acid-base balance or ketosis which occurs on a ketogenic diet but since similar results are found in rabbits after thyroid feeding in whom there is no disturbance of such a kind we are inclined to think that the lessened response is due to the depletion of liver glycogen. It is unlikely that this lessening in the response to adrenaline during a ketosis involves the muscles to any extent since it is the liver which controls the sugar content of the blood as was shown by Bollman, Mann and Magath.(41)

Additional evidence of there being such a depletion of liver glycogen on a high fat diet is presented in the following results after the injection of insulin though there will first of all be described a series of results following the injection of pituitrin.

Part IV.

Blood Sugar Curves after Pituitrin Injection.

Since pituitrin produces a hyperglycaemia somewhat similar to that of adrenaline, though of a lesser degree, it was/

TABLE V111.

BLOOD SUGAR CURVES AFTER PITUITRIN.

Case.	Age Yrs.	CCs. of Pituitrin Insect.	DIET.	Percentage (mgs.) Of Blood Sugar. Fasting Level	Minutes after Pituitrin. 10.	30.	60.	90.	120.	Rise in Blood Sugar Mgs.	URINE.
1.	12	.5	D.	.094	.107	.097	.097	.104	-	13	nil.
			F.	.081	.077	.085	.083	.092	.087	8	Acetone +
11.	10.	.5	O.	.087	.107	.107	.087	.094	-	20	nil.
			F.	.079	.094	.090	.090	.092	.094	15.	Acetone +
111.	10.	.5	O.	.090	.097	.092	.097	.092	-	-	Nil.
			F.	.087	.087	.092	.079	.077	-	5	Acetone +
1V.	11.	.5	O.	.089	.102	.113	.085	.093	-	17	Nil.
			F.	.081	.094	.104	.090	.081	.086	23	Acetone +
V.	12.	.5	O.	.106	.113	.137	.133	.135	.133	31	Nil.
			F.	.084	.104	.100	.100	.094	.094	20	Acetone +
V1.	11.	.5	O.	.100	.103	.105	.090	-	.100	5	Nil
			F.	.085	.077	.094	.089	.100	.085	15	Acetone +
V11.	9.	.5	O.	.104	.109	.115	.113	-	.098	11	Nil
			F.	.067	.094	.097	.089	.079	-	30	Acetone +
Composite Curve.			O.	.095	.105	.109	.100	.103	.110	14.	Nil
			F.	.080	.089	.095	.089	.088	.085	15.	Acetone.

BLOOD SUGAR CURVES AFTER PITUITRIN.

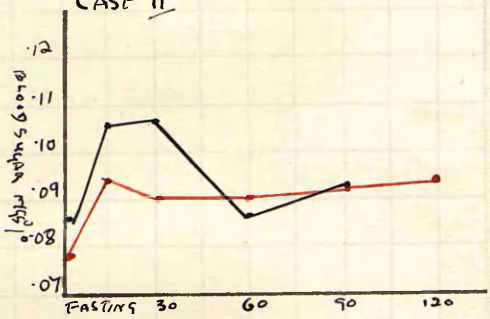
ORDINARY DIET. =

KETOGENIC DIET =

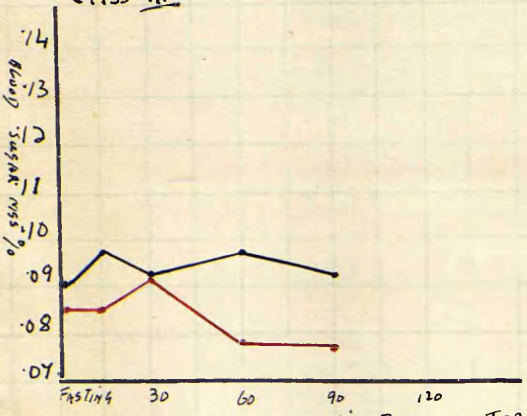
CHART IV a.

CASE I

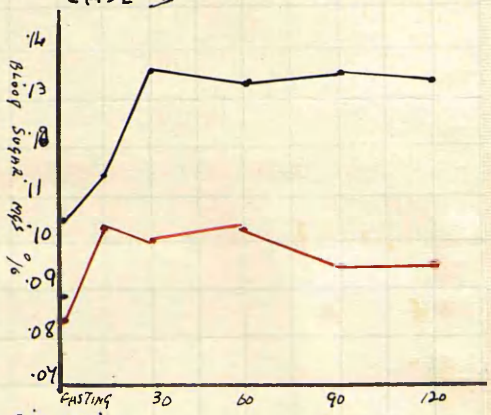
CASE II



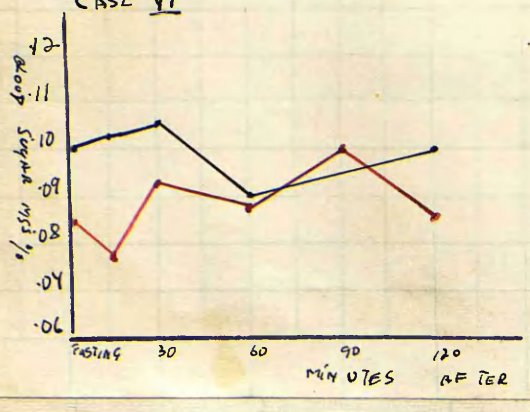
CASE III



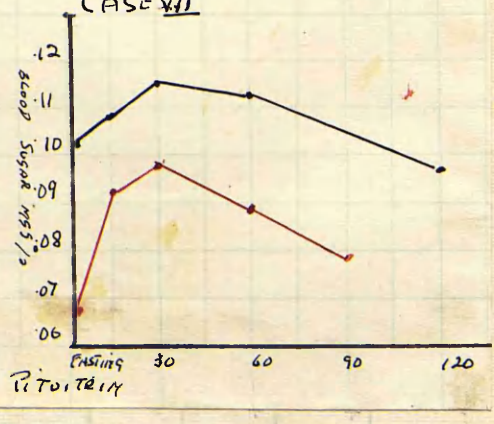
CASE V



CASE VI



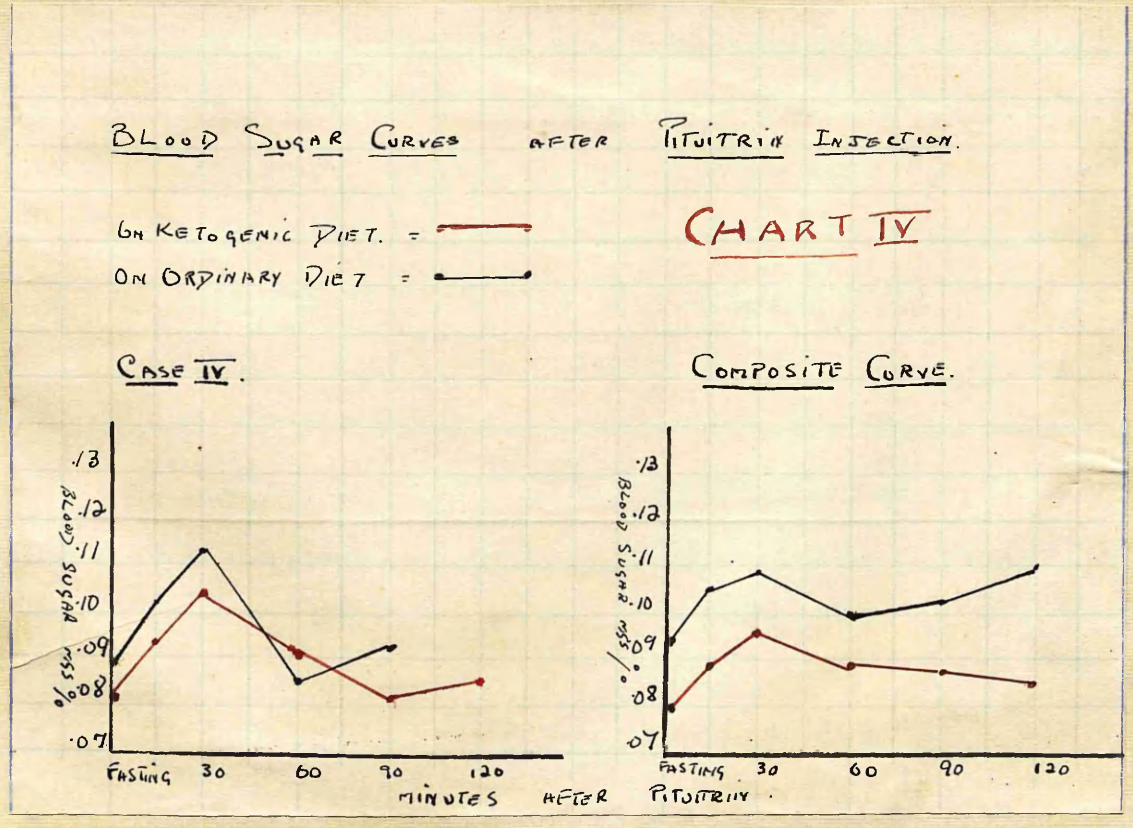
CASE VII



was decided to perform the same experiment as above, giving the subjects .5cc of "Infundin" (B.W.& Co) hypodermically and taking the blood sugar at fasting and 10, 30, 60, 90 and 120 minutes after the injection of the pituitrin. The experiment was carried out on

A. Normal Diet, and
B. "Ketogenic" Diet.

The results are tabulated in Table VIII and a representative curve and a composite curve shown in Chart IV, the remainder of the graphs being found in Chart IVa.



The hyperglycaemic effect was not so marked as that of adrenaline and in two of the cases on normal diet produced no rise in the blood sugar level. It was noticed that in every one of the subjects marked pallor of the skin was produced and all complained of cramp-like abdominal pains. These symptoms lasted for at least thirty minutes after the injection.

In cases III and VI no hyperglycaemia was produced. In cases I, IV and VII the rise, though slight, was quite definite. In case II the hyperglycaemia at 10 and 30 minutes was marked but the fasting level had been regained by one hour. Case V gave a prolonged hyperglycaemic reaction. This case reacted well to adrenaline as has already been noted.

On normal diet, therefore, only a slight hyperglycaemia occurs after the injection of .5cc of pituitrin.

On ketogenic diet the response is very similar, the difference being that the level of the blood sugar is lower but the percentage rise is practically the same. In cases I and III there was no definite hyperglycaemia. Case III reacted not at all to pituitrin on either diet and a similar "tolerance" to insulin was drawn. This is in marked contrast to case V who responded well to adrenalin, insulin and pituitrin. Both these children gave similar responses to the ingestion of glucose and there seemed no apparent reason

why they should differ so markedly in their response to these endocrine substances.

In cases II and V, the rise in slightly less than on normal diet but in cases IV, VI and VII the hyperglycaemia is slightly more marked on ketogenic diet.

A ketogenic diet therefore does not seem to affect in any way the action of pituitrin.

Part V

Sugar Toberance after "Pituitrin Feeding".

By the term "pituitrin feeding" is meant the giving of .5cc of Infundin four times daily for two days to the subject of the experiment and on the morning of the third day an amount of glucose equal to 1 grm. of glucose per kgrm. of body weight is given and the blood sugar estimated at fasting and ½, 1. 1½, and 2 hour periods after the injection. As the "pituitrin curve" was estimated the afternoon previous to the day upon which this "pituitrin feeding" process was commenced, it meant that the child received over a period of 60 hours 4.5cc of pituitrin subcutaneously. This process was carried out on

A. A Normal Diet, amd
B. A "Ketogenic" Diet.

The results are tabulated in Table IX. Chart V shows a representative case and the composite curve. For comparison the glucose curves without "pituitrin feeding" are also given.

TABLE 1X.

BLOOD SUGAR CURVES AFTER GLUCOSE
on "Pituitrin Feeding"

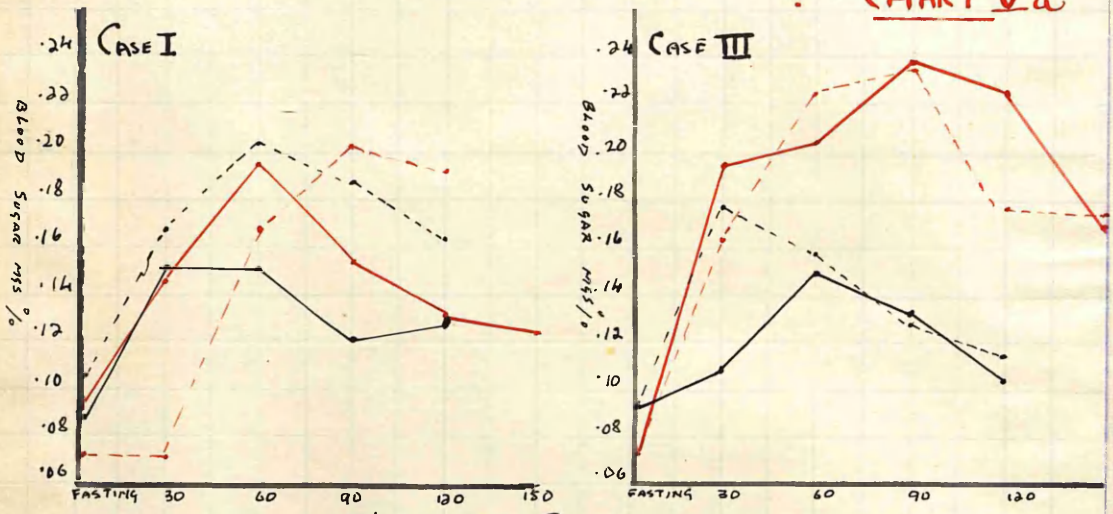
CASE.	Age Yrs.	Glucose after Dit. gms.	DIET.	Percentage (mgs) Of Blood Sugar. Fasting Level.	Minutes after Glucose. 30, 60, 90, 120, 150.	URINE.
1.	12.	28.	O.	.089	.152 .152 .120 .127	Nil.
			F.	.094	.146 .196 .156 .131 .122	Acetone +
11.	10.	27.	O.	.090	.090 .130 .122 .111 -	Nil.
			F.	.094	.152 .211 .206 .187 .177	Acetone +
111.	10.	22.	O.	.094	.109 .150 .136 .102 -	Nil.
			F.	.077	.196 .206 .236 .226 .172	Acetone +
1V.	11.	28.	O.	.089	.146 .142 .135 .100 -	Nil.
			F.	.069	.129 .168 .177 .129 -	Acetone +
V.	12.	24.	O.	.113	.184 .189 .129 .103 -	Nil.
			F.	.100	.164 .189 .187 .179 -	Acetone +
V1.	11.	27.	O.	.100	.113 .103 .104 - -	Nil.
			F.	.089	.104 .139 .129 .097 -	Acetone. *
V11.	9.	25.	O.	.109	.162 .147 .129 .097 -	Nil.
			F.	.081	.147 .214 .162 .122 -	Acetone. +++
COMPOSITE CURVES			O.	.098	.136 .145 .125 .107 -	
			F.	.086	.148 .189 .179 .155 -	Acetone +

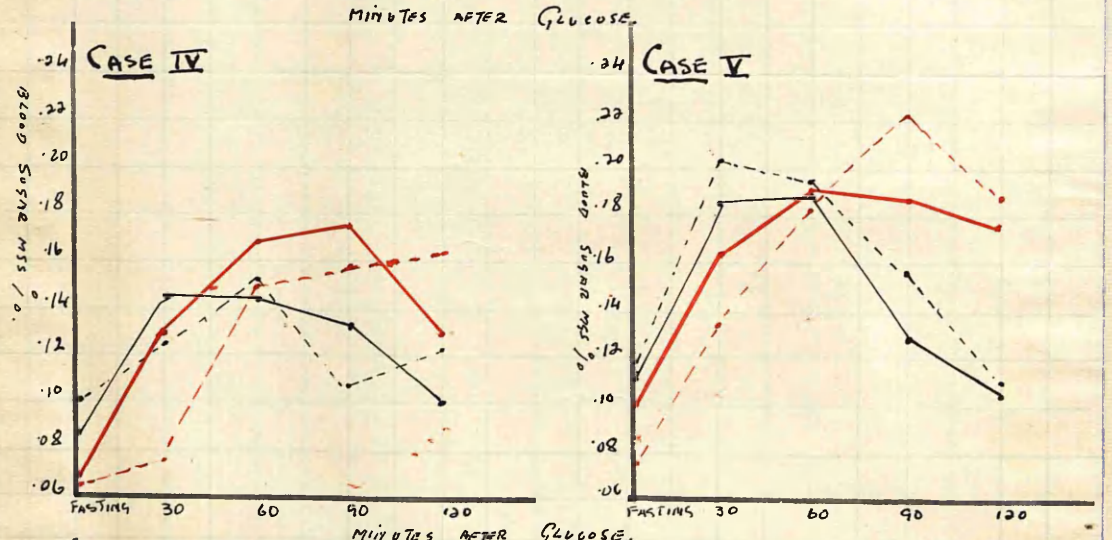
BLOOD SUGAR CURVES AFTER GLUCOSE ON "PITUITRIN FEEDING"

ORDINARY DIET: ——— AND WITHOUT PITUITRIN: - - - - -

KETOGENIC DIET: ——— AND WITHOUT PITUITRIN: - - - - -

CHART Va





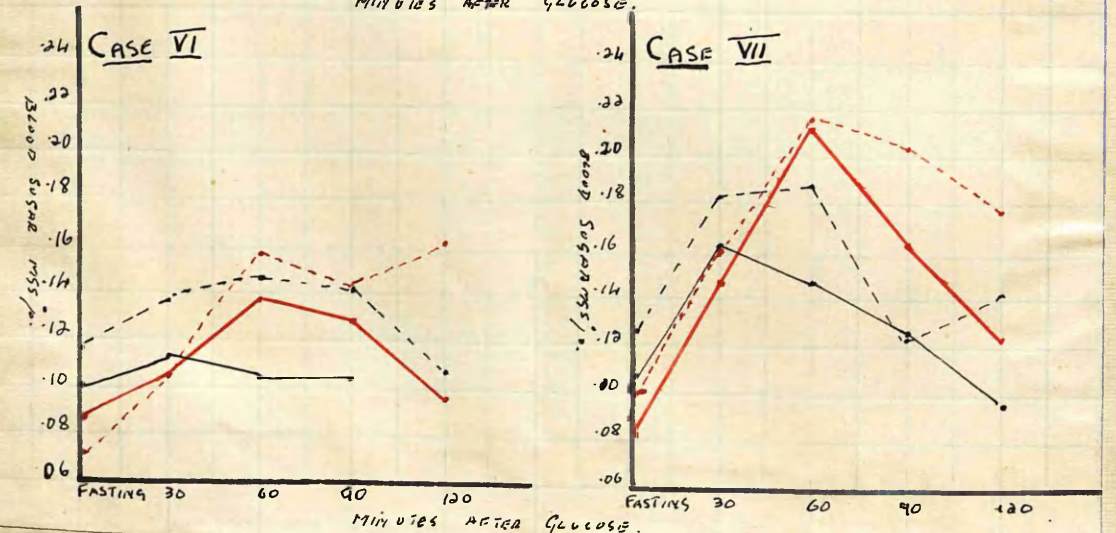
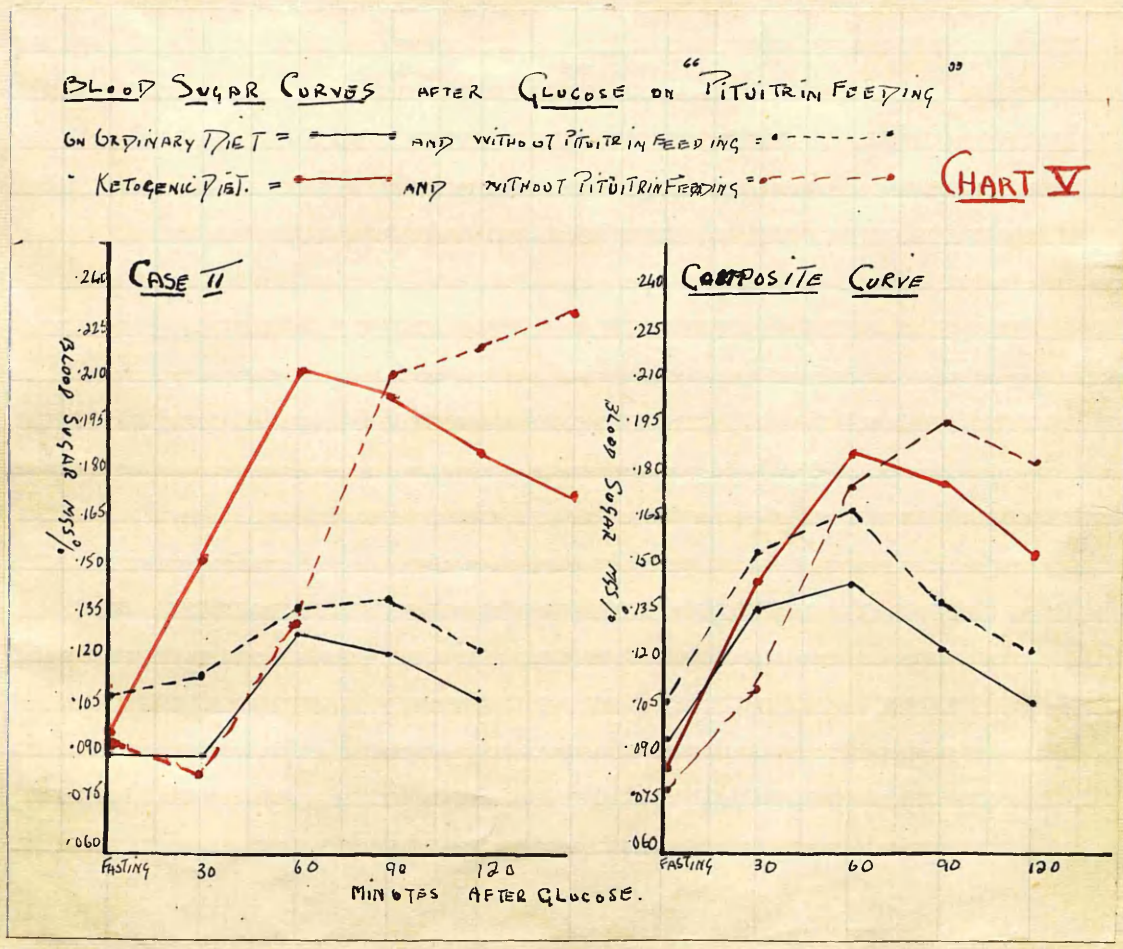


Chart V.



The effect on the tolerance for glucose is interesting In every one of the cases the curve was lower, the maximum blood sugar value being less than that where no pituitrin had been administered, and the value at the end of the two hours was nearer the fasting level.

One/

One case, A.H., not included in this work, showed a blood sugar curve with 22 grm. of glucose as follows,

F.B.S.	½hr.	1hr.	1½hr.	2hr.
.113%	.201%	.208%	.166%	.109%

and glycosuria was present. He was given .5cc pituitrin four times daily for three days and the test repeated with the following result.

F.B.S.	½hr.	1hr.	1½hr.	2hr.
.085%	.162%	.184%	.103%	.053%

and no sugar appeared in the urine.

Though the results detailed above show that the difference is slight yet it is consistent and it seems as though the injection of pituitrin had increased the tolerance for glucose.

On a ketogenic diet much the same sort of result was obtained. In case I at two hours the curve was still at .193% while after "pituitrin feeding" it had fallen to .131%. In case II the blood sugar was still rising at the two hour period, being .231% whereas after pituitrin the value at the end of the test was .177% only. In cases IV, V and VII the results are somewhat similar. In case VI the curve after "pituitrin feeding" on high fat diet is lower than that obtained after glucose alone on a normal diet.

The injection of pituitrin, therefore, over a short period/

period increases the tolerance for sugar and this effect is just as marked on a ketogenic diet as on a normal diet.

Discussion.

Mann and Magath (36) have demonstrated that there is no rise in blood sugar after the injection of pituitrin if the liver is removed and Clark (42) has shown that the muscles are not the cause of pituitrin hyperglycaemia. The source of the increase in the blood sugar content, therefore, would seem to be the liver. It is well known that pituitrin, given to a patient suffering from insulin overdosage, produces the same effect as an injection of adrenalin; a relief in the symptoms of hypoglycaemia and an increase in the content of the blood sugar. One might conclude on these grounds that pituitrin produced an increase in liver glycogenolysis. If this were so and the reaction was similar to that of adrenalin, one would expect a change in the response such as occurs with adrenalin during a ketogenic diet. No such change in response can be demonstrated. Some other factor must control pituitrin hyperglycaemia than the glycogen content of the liver.

A reasonable explanation to explain why "pituitrin feeding" should increase the sugar tolerance does not present itself. It might have been expected since a high fat/

fat diet caused a decrease in sugar tolerance that "pituitrin feeding" would have enhanced this effect since Coope and Chamberlain (43) showed that pituitrin increased markedly the fatty acid of the liver, but this was not so. McKinlay (44) demonstrated that pituitrin increased the basal metabolism and this may have some bearing on the increased ability of the tissues to utilise glucose.

Blottner and Titz (34) suggested that pituitrin molibised insulin. Stenström (45) and Burn (46) showed that pituitrin prevented the hyperglycaemia of adrenaline and this antagonism might be due to pituitrin mobilising insulin. If therefore, pituitrin increased the production of insulin the increase in sugar tolerance might be the result of an increased utilisation by the peripheral tissues of glucose, brought about by this increase in insulin. Since it is the muscles which are concerned in this increased utilisation of sugar there would be no difference in response while on a ketogenic diet which as adrenaline and insulin (in the following section) have ~~drawn~~ shown chiefly seems to affect the function of the liver.

This hypothesis might be a reasonable one if it were known only that pituitrin antagonised adrenaline and mobilised insulin but the contradictory fact that pituitrin also antagonises insulin action has been demonstrated by Burn (46)

Burn[46] and this work has been confirmed by others. There is also the clinical fact that pituitrin counteracts the effect of insulin overdosage in hypoglycaemic attacks. Coope[47] showed that the increase in the fatty acid of the liver after the injection of pituitrin could be prevented if insulin were also injected. Lambie[48] and Clark[42] demonstrated that if the liver were excluded this antagonistic action of pituitrin did not exist, the liver being necessary for its action, though this result may have been due to the fact that the removal of the liver produces hypoglycaemia in any case.

Pituitrin inhibits the action of insulin, an action similar to that of adrenalin, yet at the same time it inhibits the action of adrenalin when injected along with it. No theory can be elaborated which explains these contradictory facts.

Part VI.

Blood Sugar Curves after the Injection of Insulin.

The result recorded after the injection of adrenaline while the subjects of the experiment were receiving a ketogenic diet suggests that there is a decrease in the deposition of glycogen in the liver and it is interesting to view the results of insulin administration in the light of this hypothesis.

The/

TABLE X.

B. S. CURVES AFTER INSULIN.

CASE.	Age Yrs.	Units of Insulin	DIET	Percentage (mgs.) of Blood Sugar. Fasting Level	Minutes after Insulin 10.	30.	60.	90.	120.	150.	Fall in Blood Sugar	URINE.
1.	12.	5.	O.	.104	.094	.100	.094	.-	.089	-	10 mgs.	Nil.
			F.	.075	.060	.042	.042	.040	.064	-	35 "	Acetone ++
11.	10.	5.	O.	.107	.104	.087	.100	.109	-	-	20 "	Nil.
			F.	.102	.083	.065	.069	.053	.055	.030	72 " ∓	Acetone ++
111.	10.	5.	O.	.103	.125	.100	.094	.100	-	-	Rise 22	Nil
			O.	.082	.084	.075	.097	.072	.084	-	7[illegible] mgs.?	
			F.	.067	.063	.067	.056	.063	.063	-	11	Acetone +
			F.	.067	.077	.066	.058	.065	-	-	10?	Acetone +
1V.	11.	5.	O.	.090	.117	.104	.o90	.094	-	-	Rise 27	Nil
			O.	.104	.102	.067	.082	.082	-	-	37	Nil
			F.	.100	.091	.081	.077	.055	.069	-	45	Acetone.+
V.	12.	5.	O.	.116	.100	.087	.089	.094	.104	-	29	Nil
			F.	.092	.094	.072	.077	.075	.075	-	20	Acetone +
V1.	11.	5.	O.	.082	.089	.084	.077	.082	-	-	5	Nil
			F.	.081	.072	.042	.042	.035			46 ∓	Acetone +
V11.	9.	5.	O.	.089	.089	.077	.089	.085	-	-	12	Nil
			F.	.064	.058	.053	.052	.048	.043	-	21 ∓	Acetone +
COMPOSITE CURVES			O.	.097	.100	.086	.090	.090	.092	-	9	Nil
			F.	.081	.075	.059	.059	.054	.061	-	27	Acetone.

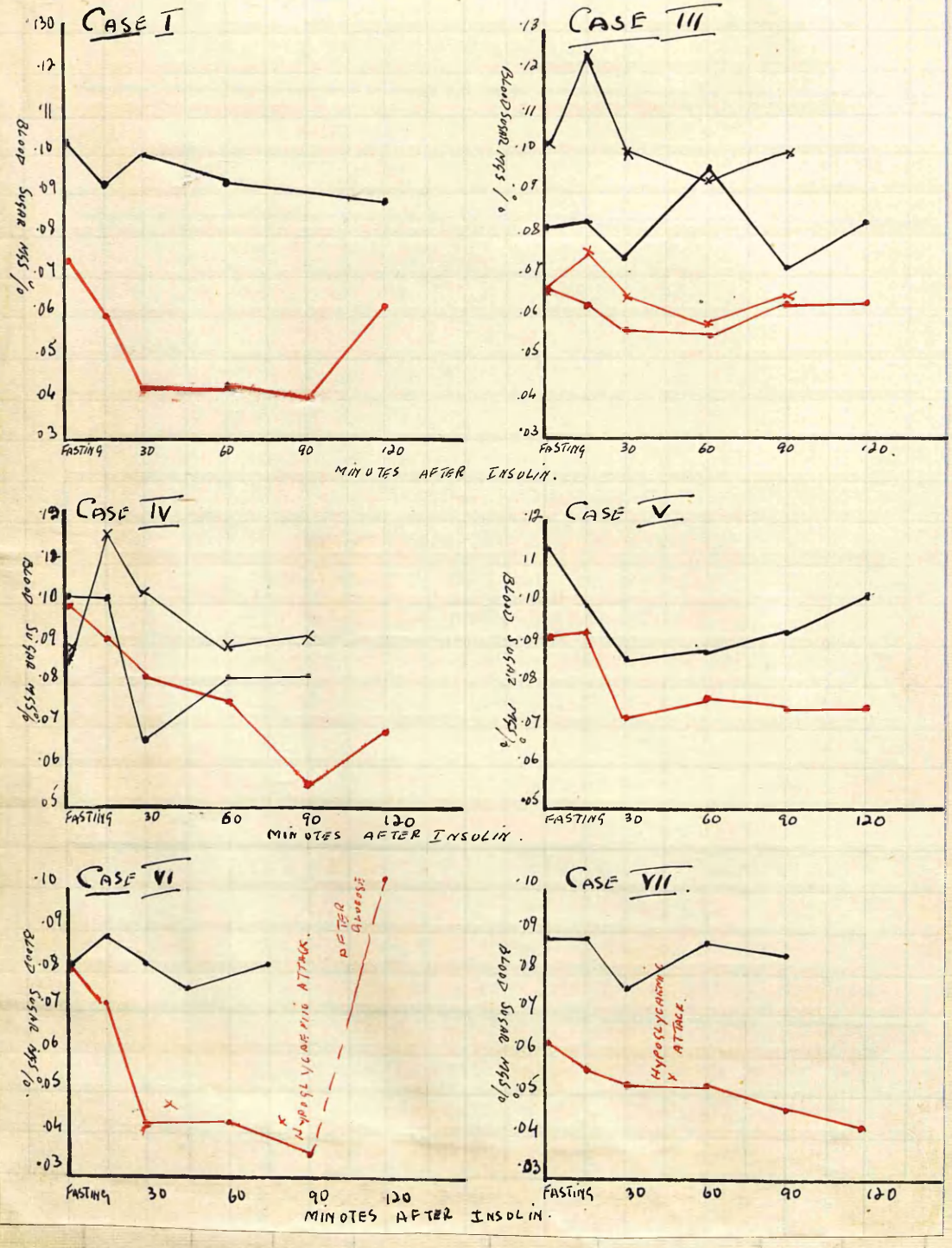
∓ :- Hypoglycaemic symptoms

BLOOD SUGAR CURVES AFTER **INSULIN.**

ORDINARY DIET =

KETOGENIC DIET =

CHART VIa

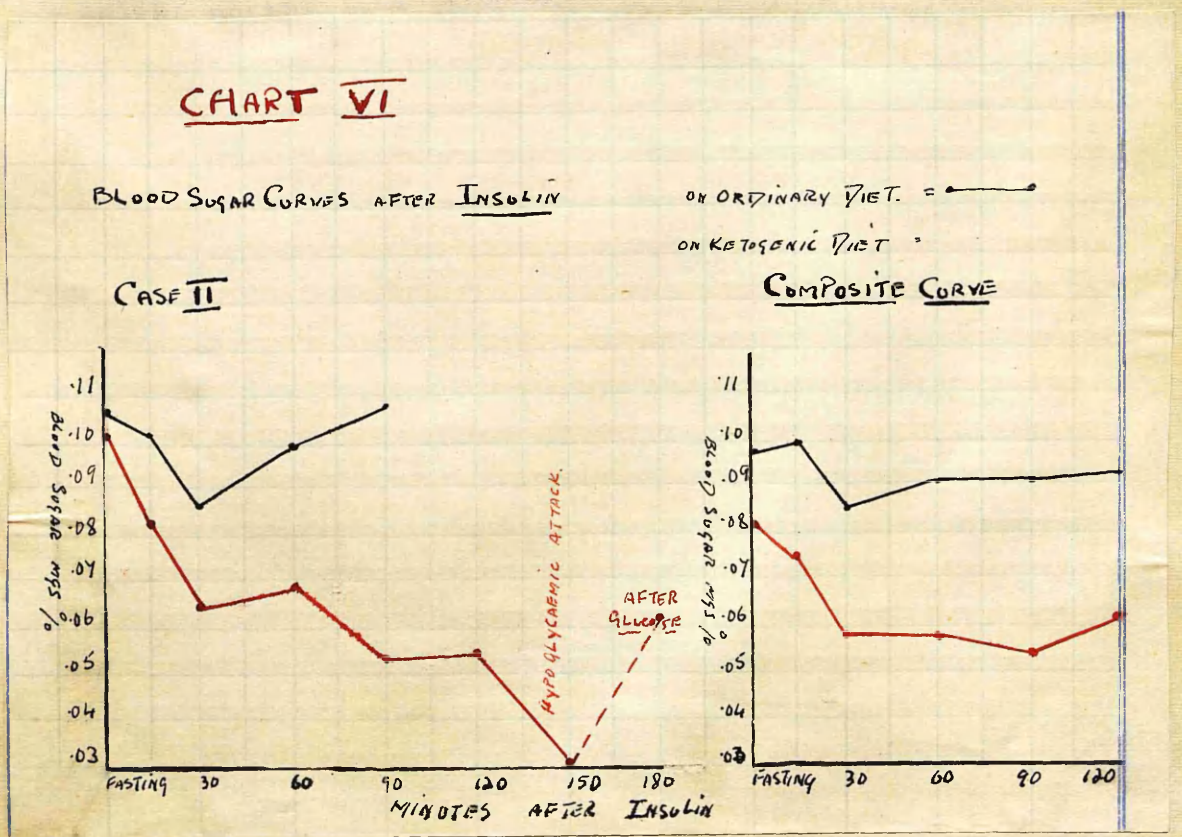


The dose of insulin throughout the experiments was 5 units (A.B.brand, B.D.H.) and samples of blood were taken at fasting and at 10, 30, 60, 90 and 120 minutes after the injection. The investigation was carried out while the children were on

A. Normal Diet, and
B. "Ketogenic" Diet.

The results are tabulated in Table X and one representative curve and the composite curve shown graphically in Chart VI. The remainder of the cases are shown in the graphs in Chart VIa.

Chart VI.



On normal diet 5 units of insulin produced a very slight hyperglycaemic reaction. In cases I and VI there was practically no change, a drop in value of 10 and 5 mgs. of blood sugar only being noted, variations which lie within the range of experimental error. Cases II, V and VII showed a more definite response, the maximum fall occurring at the ½ hour and values thereafter rising again to fasting level in 1½ and 2 hours.

Cases III and IV did not react to insulin in the manner of the other subjects in this experiment. A rise of 22 mgs. in case III was obtained with an injection of 5 units; on the test being repeated two days later a very irregular curve was the result, showing at the ½ hour a drop in value of 7 mgs. in the blood sugar and at the hour period a rise of 15 mgs. In case IV there was a rise in blood sugar of 27 mgs. 10 minutes after the insulin injection and the curve did not return to fasting level till one hour. The second test however produced the normal response, a drop in the blood sugar of 37 mgs. being obtained. This subject, as has been noted above, gave a similar reversal of results with the adrenalin injection while on a ketogenic diet, though the response was normal on a second injection being given four days later. The significance of these variations, if any significance at all can be attached to them/

them, has been discussed in the previous section.

Burn and Marks (39) found that the hyperglycaemia after adrenalin varies widely in different rabbits as does also insulin hypoglycaemia. They demonstrated that in those rabbits showing a marked hyperglycaemic response to adrenalin, the hypoglycaemia produced by insulin is less, a result which they consider to be due to adrenalin stimulating the glycogenolytic function, and the glycogenolysis so produced causing a decrease in the insulin hypoglycaemia.

In these results no such correlation is found. In cases I and VI, where insulin had but little effect, the rise obtained with adrenaline was less marked than in any of the other cases. In case V insulin produced a well marked hypoglycaemic reaction yet the hyperglycaemia produced by the adrenalin was much above the average.

If the reaction to insulin on normal diet was not striking the response after a ketogenic diet was quite different. In all of the cases but one the hypoglycaemic reaction was marked and in three of the cases definite symptoms of hypoglycaemia were noted.

The duration of time the subject was on the ketogenic diet had but little bearing on the result. Cases V and

VII/

VII were five days, I and II, six days, VI, eight days, and IV, twelve days and the insulin had much the same effect upon each. Case IV gave two practically parallel curves, though one was performed on the seventh day and the other on the twelfth day.

The level of the fasting blood sugar seemed to have little influence on the resultant hypoglycaemia as is shown by the following figures.

Case	Fasting Blood Sugar	Lowest value.	Case	Fasting Blood sugar	Lowest value.
I.	.075%	.040%	V.	.092%	.072%
II.	.102%	.030%	VI.	.081%	.035%
III.	.067%	.056%	VII.	.064%	.043%
IV.	.100%	.055%			

This was commented upon by MacLeod (50) who demonstrated that in rabbits the level of the blood sugar existing at the time of the injection of insulin and the steepness of the initial fall in blood sugar are not related, unless the level be very high when the descent becomes somewhat more rapid.

Case III, who reacted but slightly, seemed to have a tolerance for insulin shared by none of the others, and the/

the response elicited was very slight. Similar indefinite results have been recorded with this case while on a normal diet.

In case I the blood sugar fell to .042% in ½ an hour and remained at that level till the 1½ hour period when it had reached a value of .040%. The two hour sample of blood sugar was .064%. The subject showed no signs, and complained of none of the symptoms of hypoglycaemia. The maximum effect of the insulin in case V occurs at the 1½ hour period, the blood sugar value being .055%. The actual fall in the blood sugar in case V was only 20 mgs. but this lower value persisted till the 2 hour period and the curve showed no tendency to regain the fasting level, the effect being much more prolonged than on normal diet.

In case II there was a sharp drop to .065% at the ½ hour but the blood sugar continued to fall and 2½ hours after the injection of insulin the value was only .030%. About ¼ hour before this last specimen of blood was taken the child became very flushed, emotional, and complained of severe frontal headache and lassitude. She was given 20 grm. of glucose and her evening meal and in 30 minutes the blood sugar was .060% and the child was quite well again.

Case/

Case VI showed a similar sensitiveness to insulin. The blood sugar fell rapidly to .042% at the ½ hour and continued to fall till at the 1½ hour period the value was .035%. At the ½ hour or thereabouts, the boy became drowsy, pale, perspired freely and complained of "feeling queer". This lasted for one hour, the symptoms remaining the same and not increasing in severity until the 1½ hour period when glucose had to be given. In ½ hour the blood sugar had risen to .104% and he was quite recovered.

In case VII the blood sugar fell to .055% at the ½ hour and continued to fall till at 2 hours the value .043% was reached. Between the ½ hour and the 1 hour period the boy complained of "feeling ill"; he was very flushed and perspired freely. By the 1½ hour period he had recovered and said he was, and seemed to be, quite well, though the blood sugar value was actually lower than before.

From a study of these cases conclusions may be drawn regarding the level of blood sugar at which hypoglycaemic symptoms occur. Case I showed no symptoms though the blood sugar was between .042% and .040% for at least one hour. In case VI symptoms were manifest when the value .042. was reached but increased in severity at the level of/

of .035%. In case II symptoms appeared near the value .030%. Case VII had symptoms with a blood sugar content of .053% but these had cleared up when the value was .043%, i.e. lower than before.

It would seem that the blood sugar level at which hypoglycaemic symptoms supervene lies somewhere near .040% and .050%. MacLeod et al[51] found that in rabbits hypoglycaemic convulsions occurred when the blood sugar level was, on the average, .045%; the lowest level at which no convulsions occurred being .037% and the highest level at which a convulsion occurred being .063%. Mann and Magath[52] also found that in dogs the hypoglycaemic level varied between .060% and .030%. Talbot, Shaw and Moriarty[32] report, in epileptics who were fasted for 10-14 days, blood sugar levels of an average value of .046%. One of their cases with acidotic symptoms had a blood sugar content of .038%. Weymuller and Schloss[33] record blood sugar values as low as .042% and .035% without any symptoms of hypoglycaemia. Two diabetic children, under treatment in the Royal Hospital for Sick Childre[n], while suffering from hypoglycaemic symptoms showed blood sugar values of .037%. One of the children, who frequently took hypoglycaemic convulsions always registered this low level during/

during the attack. Widnas (21) reports in a diabetic a blood sugar of .038% without any hypoglycaemic symptoms. As already reported in this paper, a child after an eight hour fast had a blood sugar content of .040% on two occasions and was not disturbed in any way.

Some workers have considered that some of the symptoms of cyclical vomiting and of acidosis are due to the low blood sugar and strength is given to this view because the administration of glucose in these conditions relieves the symptoms. It can be seen from the above however that though a low blood sugar may produce a "hypoglycaemic" attack there are other occasions when the sugar content of the blood is very low and yet no symptoms supervene. Some other factor would seem to be necessary, combined with the lowering of the blood sugar, before manifest symptoms are produced.

Discussion.

It is obvious from the above results that while on a ketogenic diet the hypoglycaemic action of insulin is accentuated and that there is only a slight tendency for the blood sugar to regain its normal fasting level. Is a decrease in the glycogen content of the liver sufficient to account for this?

When insulin was given to depancreatised dogs it was found that glycogen was deposited in the liver and it was thought/

thought, therefore, that the liver under the influence of insulin removed the sugar from the blood stream and converted it into glycogen. Dudley and Marrian[53] and Babkin[54] found, however, that rabbits fed on carrots and then injected with insulin, showed a reduction of glycogen in the liver and skeletal muscles. Fisher and Lackey[55] demonstrated similar results in dogs. Others have worked at this problem and found more or less the same results and so far no one has conclusively demonstrated that glycogen formation in the liver is primarily a factor in causing the fall in blood sugar after insulin administration. Whatever the cause of the increased response to insulin while on a ketogenic diet it is not likely to be due to a stimulation of the glycogenic function of the liver.

Further research was done by various workers on the problem of what happened in the normal animal when insulin was given and Burn and Dale[56] showed that the skeletal muscles are largely responsible for the disappearance of dextrose under insulin. This was confirmed by Best, Hoet and Marks.[57] Cori and Cori[58] found that along with the decrease in liver glycogen there was an increase in sugar oxidation and later they[59] came to the conclusion that the decrease in deposition of glycogen in the liver under the action of insulin was due to an increase in the utilisation of/

of absorbed sugar in the peripheral tissues. Macleod[60] and his co-workers have shown that during the active absorption of carbohydrate large amounts of insulin cause glycogen to be deposited in the muscles this being accompanied by a corresponding decrease in the amount deposited in the liver. Mann and Macgath[61] demonstrated that the presence of the liver was not necessary for the hypoglycaemic action of insulin although they did not consider that this precluded the liver from being indirectly involved in the action.

It might be, therefore, that the peripheral tissues, under the influence of a ketosis or acidosis absorb more sugar from the blood stream than normally and so prevents the return of the blood sugar to a normal fasting value. That this is an unlikely explanation is shown by the reaction of the organism to glucose, the hyperglycaemia being marked and prolonged, not reduced as it would be were the tissues utilising sugar at a quicker rate than usual.

The change in the acid base equilibrium which is produced by an effective ketogenic diet might quite possibly have an effect on the response to insulin but it is interesting to note in this connection that Page[62] fed rabbits on an acid forming diet and found that they were more resistant to insulin. This acid forming diet caused

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a rich deposit of glycogen in the liver so it would seem that the amount of glycogen in the liver was of more importance than the disturbance in acid-base equilibrium. Also there is no ketosis accompanying the latter disturbance in acid-base balance as there is in the acidosis of a ketogenic diet. If the results reported here are due to a depletion of glycogen in the liver, is the ketosis the important factor, and not the acidosis, in producing this depletion?

Is there, then, a marked reduction in the deposition of glycogen in the liver? Mann and Magath (61) have shown that the hypoglycaemic action of insulin does not depend primarily upon the liver but that unless the liver is present no recovery of blood sugar occurs. That it is not the muscles which contribute to this recovery after insulin hypoglycaemia was demonstrated by Ballman, Mann and Macgath (41) who proved that the glycogen of the muscles is incapable of rapid conversion to glucose to maintain the level of sugar in the blood. Best, Haet and Marks (57) demonstrated that no decrease of muscle glycogen occurred in insulin hypoglycaemia unless severe convulsions took place. That the prolonged action of insulin is not due to any inhibition, by the ketosis or acidosis of the adrenals was shown by Stewart and Rogoff (63) who found that

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the action of insulin on rabbits which had survived the complete removal of the adrenals was the same as in the normal animal.

In the light of this knowledge the results of the above experiments would seem to point to there being a deficiency in the glycogen stores of the liver and that this is the reason for the increased and prolonged insulin action. Others workers, who have investigated this problem carried out their experiments on animals and were in a position to estimate the glycogen content of the liver. Macleod et alia (64) found that in rabbits, the richer the glycogen content of the liver the more resistant to insulin they were. Burn and Marks (39) demonstrated that after feeding thyroid for 18 days to rabbits they showed an increased sensitiveness to insulin and on estimating the glycogen content of the livers it was found to be reduced. Raper and Smith (65) also found in decerebrate cats, from which the pituitary body had been removed, that insulin produced a more marked effect when the glycogen of the liver was reduced. Lawrence (66) has also pointed out that in patients suffering from diabetes mellitus, insulin is much more effective when the glycogen stores are empty.

The conclusion can therefore be come to that whatever the/

the cause there is a depletion of glycogen in the liver. Blatherwick and his co-workers (67) have shown that an acid forming high carbohydrate diet can lay down glycogen in the liver and muscles so that it does not appear as though the acidosis per se interfered with the glycogenic action of the liver and it may be that it is the ketosis which is the important factor in the disturbance of carbohydrate metabolism.

Part VII.

"The Laevulose Test"

Whatever the reason, ketogenic diet lowers the tolerance for glucose and evidence has been presented in parts III and VI to the effect that there is probably a reduction of the glycogen content of the liver. This depletion of liver glycogen may not be due to any failure on the part of the liver to perform its glycogenic function, but may occur because of the heavy demands made upon it to supply the body with the necessary carbohydrate which it fails to receive on a high fat, low carbohydrate diet. If, however, there is an actual reduction in the ability of the liver to perform its glycogenic function then one might expect the "Laevulose Test" to be positive. The Laevulose Test is based on the fact that there is no appreciable rise in

TABLE XI.

BLOOD SUGAR CURVES AFTER LAEVULOSE

CASE.	Amount of Laevul. gms.	DIET.	Percentage (mgs.) of Blood Sugar: Fasting Level	Minutes after Laevulose: 30.	60.	90.	120.	150.	Rise in Blood Sugar. %	mgs.	URINE.
1.	20	O.	.100	.109	.094	.089	-	-	9.	9.	Nil.
		F.	.095	.126	.105	.117	.111	-	32.	31.	Acetone +
2.	20	O.	.090	.100	.090	.100	.105	-	16	10	Nil.
		F.	.074	.100	.134	.117	.100	-	80	60	Acetone +
3.	28	O.	.117	.122	.149	.134	.129	-	27	32	Nil
		F.	.117	.162	.162	.171	.143	-	46	54	Tr.Sugar 1½ hrs.
4.	20	O.	.112	.100	.112	.100	-	-	-	-	Nil.
		F.	.117	.152	.134	.152	.157	-	34	40	Acetone +
5.	28	O.	.104	.113	.117	.113	.109	-	12	13	Nil
		F.	.117	.114	.137	.162	.162	.117	38	45	Acetone +
6.	23	3 O.	(.095	.109	.100	.100	.085	)3 -	54	54)	Nil
		1 O.	(.100	.154	.129	.117	.109	)1 -	6	6)	
		2 O.	(.100	.104	.090	.106	.087	)2 -	15	14)	
		F.	.072	.085	.109	.109	.100	-	51	37	Acetone +
7.	25	O.	.081	.100	.090	.072	.095	-	23	19	Nil
		F.	.077	.134	.117	.094	-	-	74	57	Acetone +
8.	23	O.	.129	.162	.147	.147	.137	-	25	33	Nil
		F.	.100	.142	.114	.123	.089	-	41	41	Acetone +
9.	19	O.	.090	.102	.104	.085	.085	-	15	14	Nil
		F.	.072	.104	.081	.077	-	-	44	32)	
		F.	.085	.085	.104	.093	.077	-	22	19)	Acetone +

TABLE XI (Continued)

CASE.	Amount of Laevul. gms.	DIET.	Percentage (mgs.) of Blood Sugar. Fasting Level	Minutes after Laevulose 30.	60.	90.	120.	150.	Rise in Blood Sugar %	mgs.	URINE.
10.	20.	O.	.012	.106	.100	.110	-	-	-	-	Nil.
		F.	.077	.090	.109	.096	.090	-	41	32	Acetone +
11.	23	O.	.111	.131	.125	.112	.100	-	18	20	Nil
		F.	.089	.113	.123	.115	.084	-	38	34	Acetone +
12.	25	O.	.097	.114	.093	.084	-	-	17	17	Nil
		F.	.084	.113	.123	.115	.084	-	40	33	Acetone +
13.	20	O.	.119	.148	.154	.116	.116	-	29	35	Nil
		F.	.063	.113	.152	.123	.072	-	141	89	Acetone +
14.	28	O.	.104	.117	.113	.100	.102	-	12	13	Nil
		F.	.077	.118	.135	.111	.106	-	75	58	Acetone +
15.	23	O.	.087	.106	.094	.085	-	-	21	19	Nil
		F.	.053	.080	.097	.092	.092	-	82	44	Acetone +
16.	30	O.	.092	.109	.104	.102	-	-	18	17	Nil
		F.	.075	.103	.137	.103	.089	-	82	62	Acetone +
17.	28	O.	.094	.104	.104	.103	.089	-	9	10	Nil
		F.	.040	.084	.106	.109	.125	-	237	95	Acetone +

the sugar content of the blood after the ingestion of laevulose in individuals in whom there is no disease of the liver. If the hyperglycaemia obtained shows a rise of over 30 mgs.% then the liver is considered to be damaged. It was considered, therefore, that some further evidence of liver disturbance might be obtained if this test were carried out on

A. A Normal Diet, and
B. A "Ketogenic" Diet.

Seventeen investigations were made. The children who were used as the subjects of the experiment varied in age from 5 to 12 years. Thirteen of the children were convalescent from chorea, two from Acute Rheumatism, and one from Empyema. One case suffered from "Petit Mal" and during the period of the experiment took frequent fits, the ketogenic diet having no effect whatever on their frequency.

To act as a control, blood sugar curves after 1 grm. of glucose per kgrm. of body weight were estimated on ordinary and on high fat diet. In one case only, case 14, the curve obtained while on a ketogenic diet resembled that on normal diet. All the others showed a reduced tolerance for glucose. The details of this work are not given since they merely confirmed the findings of the first part of this thesis.

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The children were given 1 grm. of laevulose (Merck's Pure) per kgrm. of body weight and the sugar content of the blood estimated at ½, 1, 1½ and 2 hourly intervals. The urine was examined for sugar and acetone, specimens being taken where possible immediately after the sample of blood had been removed.

The results are given in detail in Table XI.

The average percentage rise in the blood sugar after the ingestion of laevulose was only 17 mgs.% and only one of the cases, case 6, showed a rise of over 30 mgs.%. This being considered an unusual result the test was repeated on two other occasions and a rise of 6% and 15% only obtained. Taylor-Chadwick (68) has shown that occasionally cases of chorea with attendant heart lesions show an intolerance to laevulose and that this may disappear after an improvement in the general condition of the patient. This may have been the case here though there was no very definite change noted in the clinical condition of the child. In two of the cases no rise was obtained at all and in nine of the cases the increase was less than 20 mgs.%.

On a ketogenic diet there is a marked reduction in the tolerance for laevulose. The average percentage increase was 66 mgs.% and in sixteen of the seventeen cases

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a higher blood sugar curve was obtained than on a normal diet.

In case 13 the test was performed after the child had been on a ketogenic diet for two days only (a shorter period than usual) and an increase of 44 mgs.% in the blood sugar was registered. In order to discover if the longer period on ketogenic diet increased the severity of the disturbance of liver function the test was repeated four days later and a rise of 22 mgs.% only obtained, though the maximum blood sugar value was equal on both occasions and the fall was more delayed the second time.

In six of the cases the blood sugar value increased over 70 mgs.%. The actual increase in mgs. was only 85 but the percentage increase was so high because of the very low fasting blood sugar of .040%. In only one, case 3, did the value of the blood sugar rise above .170% and glycosuria was present. In none of the other cases did glycosuria arise though four of the cases showed blood sugar values above .140% which is supposed to be the renal threshold for laevulose. Graham (69) found laevulose excreted though the blood sugar value never exceeded .150% and Spence and Brett (70) found laevulosuria at levels varying from .097% to .135%. They pointed out however that the threshold seemed to vary widely in different individuals.

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In a case of jaundice, M.M., considered to be caused by a neoplasm of the liver, glycosuria occurred though the maximum value recorded was only .117%. Two months later, when his condition was much improved no glycosuria resulted though the laevulose curve reached a value of .131%.

Discussion.

Cathcart and Markowitz (71) suggested that laevulose cannot be oxidised as such but must first be converted to glucose in the liver. Mann and Magath (36) fed laevulose to liverless dogs but this did not produce any effect on the hypoglycaemia and no glucose was deposited in the muscles, as occurred after the ingestion of glucose in hepatectomised animals. Corri and Corri (72) consider that the liver is of greater importance in the removal of laevulose from the blood stream than it is in the case of glucose. The liver has first to convert the laevulose to glucose and then this glucose is utilised by the liver to form glycogen. Since the liver is of more importance in this action of glycogenesis than the peripheral tissues with respect to laevulose it seems probable that the ability of the liver to convert glucose to glycogen has become impaired and that, in consequence, the liver becomes depleted of its store of glycogen.

I consider, therefore, that, in a ketogenic diet acidosis/

acidosis, there is a depletion of glycogen in the liver and that this is due to a decrease in the capacity of the liver to deal with glucose brought to it by the blood stream.

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SECTION IV.

The Blood Sugar Curve after the Administration of Ammonium Chloride.

A "ketogenic" diet produces a non-gaseous acidosis, having a diminished alkaline reserve and an accompanying ketosis. The administration of sufficient amounts of Ammonium Chloride results in a similar acidosis but in this case there is no ketosis. Now the reduction in carbohydrate tolerance occurring with a low carbohydrate diet may have been due to the acidosis, the ketosis, or the decreased metabolism of carbohydrate failing to provide the sugar needed to enable glucose to be quickly utilised. If there is found a similar reduction in the tolerance for sugar when Ammonium Chloride is administered one would be able to conclude that it is the acidosis which is the important factor, and that the ketosis or the lack of carbohydrate had but little effect. On the other hand if no such reduction in tolerance is produced then the ketosis or the change in the composition of the diet is the important factor.

An investigation, therefore, into the tolerance for glucose and laevulose during Ammonium Chloride administration was made. The results are detailed in Tables XII and XIII

and/

TABLE XII. (Glucose)

Blood Sugar Curves after Ammonium Chloride Admt.

Case.	Admt. of Glucose.	Diet and Drugs.	No. of Days on Am.Chl.	PERCENTAGE (mgs) of Blood Sugar. Fasting Level	MINUTES AFTER GLUCOSE 30.	60.	90.	120.		URINE.
J.F.	28	Ordinary	-	.106	.166	.204	.189	.162	-	Nil.
		O+Am.Chlo.	4	.104	.144	.164	.104	.082	-	Nil.
F.F.	25	Ordinary	-	.127	.181	.189	.123	.141	-	Nil.
		O+Am.Chl.	4	.058	.154	.134	.077	.041	-	Nil.
M.G.	27	O	-	.109	.115	.137	.141	.125	-	Nil.
		M+S+Am.Chl.	7	.113	.181	.211	.172	.141	-	Sugar ++
N.C.	30	O	-	.156	.186	.147	.162	.129	-	Nil
		M+S+Am.Chl.	7	.134	.261	.206	.196	.181	-	Tr. Sugar.
J.F.	21	O	-	.077	.137	.157	.152	.113	-	Nil.
		M+S+Am.Chl.	15	.085	.134	.137	.152	.134	-	Nil.
R.H.	16	O	-	.085	.129	.160	.139	.113	-	Nil.
		O+Am. Chl.	8	.085	.209	.201	.117	.067	-	Nil.
G.McD.	26	O	-	.109	.175	.147	.134	.113	-	Nil.
		Milk Diet	-	.084	.193	.206	.150	.063	-	Nil.
		O+Am.Chl.	8	.109	.172	.234	.189	.085	-	Nil.
		M+Am.Chl.	7	.077	.184	.189	.182	.109	-	Nil.
C.McG.	25	O	-	.129	.181	.196	.141	.131	-	Nil.
		Milk	-	.100	.152	.177	.129	.104	-	Nil.
		O+Am. Chl.	8	.109	.207	.177	.122	.100	-	Nil.
		O+Am. Chl.	16	.104	.161	.157	.166	.095	-	Nil.
		M+Am. Chl.	24	.115	.209	.254	.164	.107	-	

TABLE XII. (Continued)

Case.	Admt. of Glucose	Diet and Drggs.	No. of days on Am.Chlo.	Percentage (mgs.) of Blood Sugar: Fasting Level	MINUTES AFTER GLUCOSE: 30.	60.	90.	120.		URINE.
I. J.	18.	O	-	.095	.137.	177	.116	.100	-	Nil.
		O+Am.Chl.	6	.109	.149	.152	.129	.081	-	Nil.
		O+Am.Chl.	18	.092	.141	.157	.152	.113	-	Nil.
		O+Am.Chl.	24	.113	.152	.186	.162	.117	-	Nil.
		M+Am.Chl.	32	.109	.174	.204	.194	.131	-	Nil.
		M i l k	-	.098	.132	.132	.077	.072	-	Nil.
M.McC.	20.	O	-	.109	.171	.177	.109	-	-	Nil.
		Special Diet	-	.109	.123	.166	.109	.094	-	Nil.
		Sp.Diet Am.C.	11	.094	.109	.193	.117	.129	-	Nil.
		O+Am. Chl.	23	.094	.162	.137	.147	.100	-	Nil.

BLOOD SUGAR CURVES AFTER GLUCOSE

CHART VII a

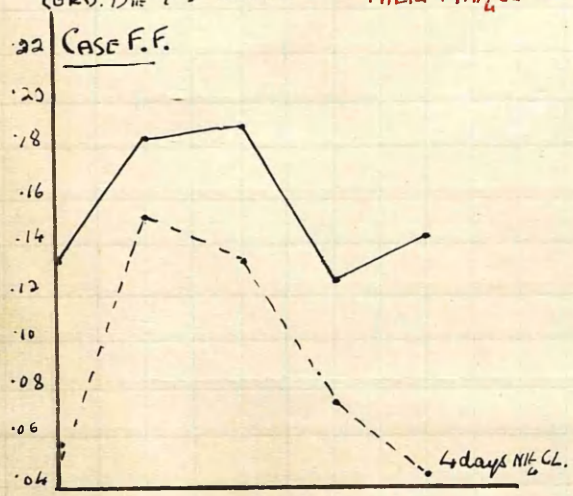
GRD. DIET = ——

MILK DIET = •- - - -•

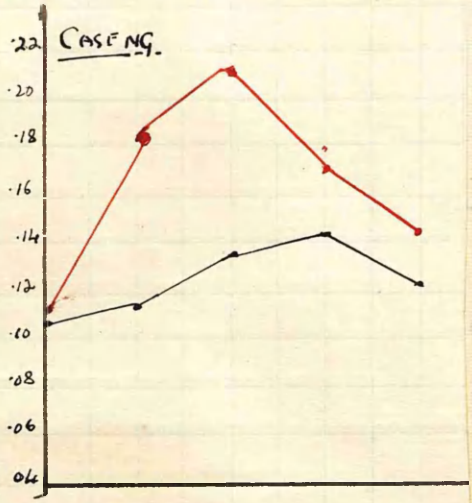
{NH₄CL + GRD. DIET = •- - - -•

MILK + NH₄CL = ——•

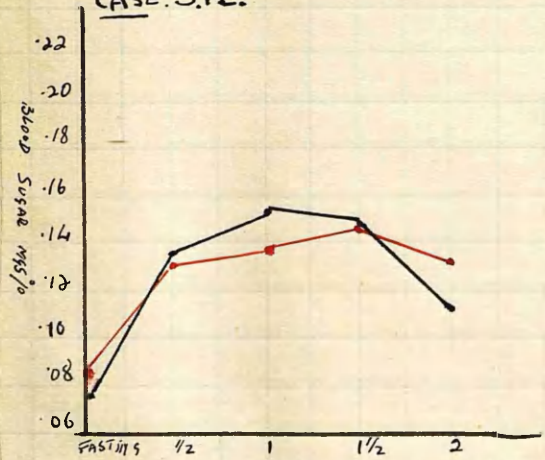
CASE F.F.



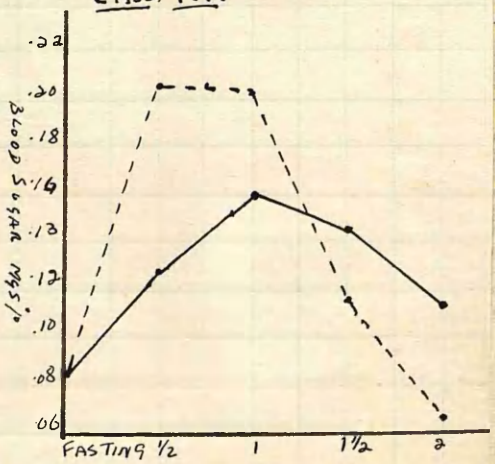
CASE N.G.



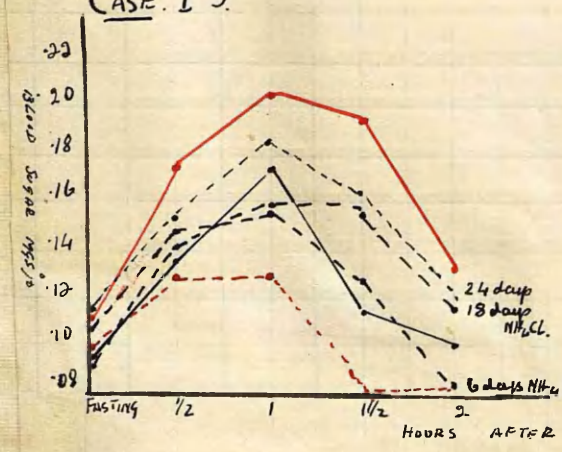
CASE. J.FE.



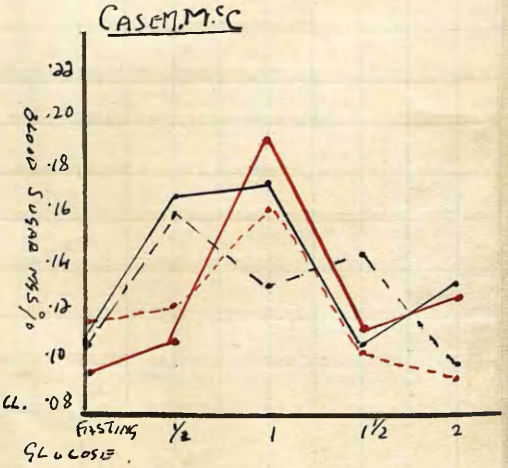
CASE. R.H.



CASE. I.J.

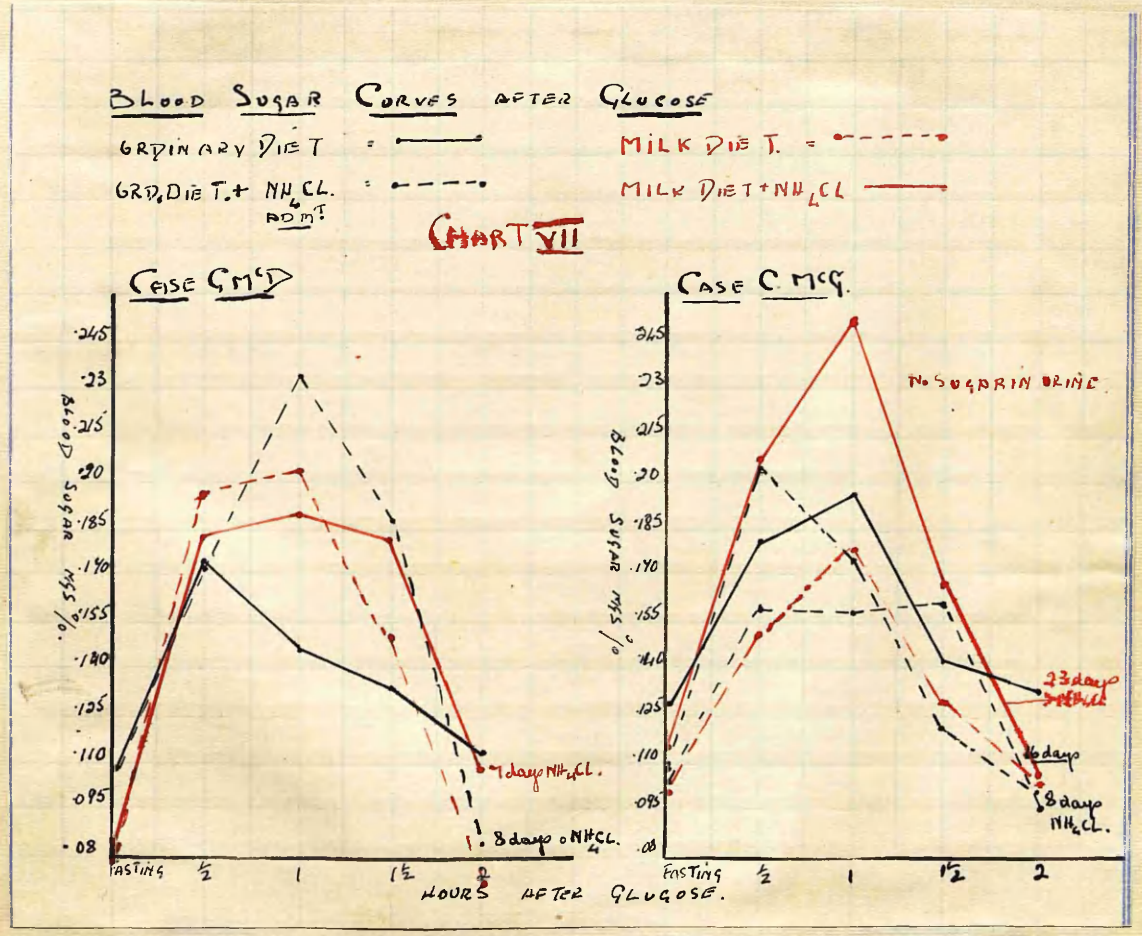


CASE M.M.C



and representative results shown graphically in Charts VII and VIII.

Chart VII.



The subjects of the investigation were nine children convalescent either from Rheumatism or Chorea. The quantity of Ammonium Chloride was calculated roughly from the amount which Haldane (73) used in his investigation into its action. All the children who were more or less the same weight were given 5 grm. of Ammonium Chloride daily. The number of days they received the drug and the diet they were given varied in each case and will be described as each one is dealt with.

Case J.F. and F.F. were given Ammonium Chloride for four days and both showed an increased tolerance for glucose, the curve being lower and the value returning sooner to fasting level than on ordinary diet. On a ketogenic diet both these children showed a reduced tolerance for glucose. That the condition of acidosis has been definitely established by the fourth day can be seen from the following table showing the change in the CO_2 content of the blood. This blood analysis was carried out by Dr. N.Morris of the biochemical department in the Royal Hospital for Sick Children and to whom I am much indebted for the use of these figures.

Case.		CO_2 Vol.%
N.C.	Normal	68.2
	4 days NH_4Cl	41.4
	8 " "	40.3

N.M./

Case.		CO_2 Vol.%
N.M.	Normal	60.6
	3 days NH_4Cl	45.1
	6 " "	41.8
	9 " "	43.4
	13 " "	41.5
J.F.	Normal	66.7
	9 days NH_4Cl	49.1
	19 " "	45.8

The acidosis in these two cases at least has not reduced the tolerance for glucose.

The next three cases, N.G., N.C. and J.F. were given for 10 days previous to the test a milk diet plus 40 grm. of sugar daily, this diet containing an adequate caloric content for their respective weights. Then for seven days in the case of N.G. and N.C. and for thirteen days in J.F., 5 grm. of Ammonium Chloride was given and then the sugar tolerance estimated. In N.G. and N.C. a markedly reduced tolerance was noted and glycosuria occurred in both cases. In the case of N.G. though glycosuria occurred the blood sugar did not show the prolongation of hyperglycaemia which was obtained on a ketogenic diet (case II in Section III of this thesis). In J.F. there was practically no change in the blood sugar values from those obtained while on ordinary diet. He showed, however, glycosuria with 145 grm. of glucose whereas normally he passed no sugar with/

with this quantity of glucose.

Cases R.H., G.McD. and C.McG. were given Ammonium Chloride for eight days and their tolerance tested. R.H. and G.McD. showed a definite reduction and C.McG. a slight reduction in tolerance. The hyperglycaemia had gone and the fasting level regained by the end of two hours. The tolerance for a larger quantity of glucose was tested in these three cases. R.H. had slight glycosuria with 112 grm. and G.McD., with 105 grm. of glucose, showed an abundant glycosuria. On ordinary diet no glycosuria resulted with these amounts of glucose. C.McG. had glycosuria with 140 grm. of glucose but unfortunately on normal diet she was unable to retain this quantity of glucose. C.McG. was kept on the drug for sixteen days and the tolerance estimated again and a lower blood sugar curve than that obtained on ordinary diet was the result. The acidosis of Ammonium Chloride persists for longer periods than this as J.F. after nineteen days showed a CO_2 vol.% of 45.8. G.McD. and C.McG. were given a milk diet, without the addition of sugar, but containing their full caloric requirements, along with the administration of Ammonium Chloride. G.McD. showed no change in tolerance but C.McG. gave a definitely higher curve than before. She had been by this time 24 days on the Ammonium Chloride.

C.McH's/

C.McG's response to glucose after ten days on a ketogenic diet showed a very marked lowering of the tolerance for glucose, glycosuria occurring after 25 grm. of glucose only and the blood sugar estimations were as follows.

F.B.S.	½hr.	1hr.	1½hr.	2hr.
.104%	.191%	.251%	.247%	.162%

much higher than the results with Ammonium Chloride.

In the next case, I.J., an attempt was made to discover if the duration of the administration of the drug had any effect. As can be seen from Table XII there is little change even after 24 days. She was given a milk diet and the test repeated again but only a slight increase in the hyperglycaemia is recorded, even after 32 days on Ammonium Chloride.

Since milk has a ratio of 1 of Protein and 1 of Fat to 1.5 of Carbohydrate this milk diet is rather low in carbohydrate compared to an ordinary mixed diet or the milk diet containing 40 grm. of sugar. In order to discover if this milk diet had any effect upon the tolerance for glucose cases G.McD., C.McG. and I.J. were given a milk diet for six to ten days and the tolerance for glucose tested. G.McD. was the only one showing a slightly increased hyperglycaemia and it was concluded that the change in diet had little effect.

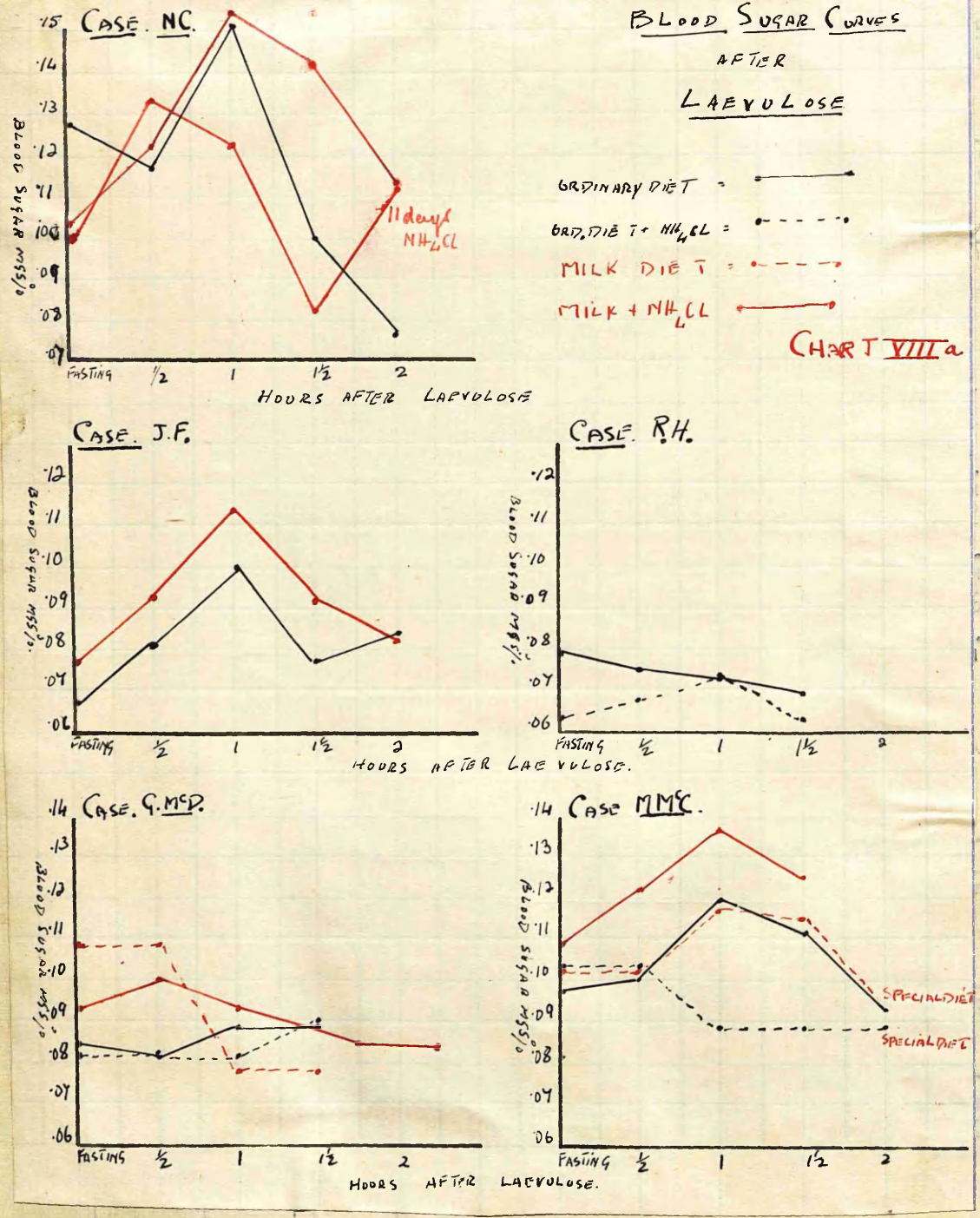
M./

TABLE XIII.

LAEVULOSE CURVES AFTER AMMONIUM CHLORIDE ADMT.

CASE.	Admt. of Laev.	Diet and Drugs.	No. of days on Am.Chl.	PERCENTAGE (mgs.) of Blood Sugar. Fasting Level	MINUTES AFTER LAEVULOSE 30.	60.	90.	120.	Rise in Blood Sugar.	URINE.
N.C.	30 grm.	O	-	.129	.117	.152	.100	.077	23 - 17%	Nil.
		M+S+Am.Ch.	11.	.100	.134	.122	.082	.113	34 - 34%	Nil.
		M+S+Am.Ch.	10.	.104	.122	.151	.141	.113	47 - 45%	Sugar.+
J.F.	21.	O	-	.068	.081	.100	.077	.086	32 - 47%	Nil.
		M+ S+Am.Ch.	11.	.077	.092	.113	.092	.082	36 - 46%	Nil.
R.H.	16.	O	-	.079	.079	.075	.072	.069	- -	Nil.
		O+ Am.Ch.	8.	.063	.068	.073	.063	-	10 - 15%	Nil.
G.McD.	26.	O	-	.085	.081	.089	.089	-	4 - 5%	Nil.
		O+Am. Ch.	13.	.082	.082	.082	.090	-	8 - 9%	Nil.
		M+Am.Chl.	7.	.094	.100	.094	.085	.087	6 - 6%	Nil.
		M	-	.109	.109	.077	.075	-	- -	Nil.
C.McG.	25	O	-	.097	.114	.093	.084	-	17 - 17%	Nil.
		O+Am.Chl.	12	.104	.115	.107	.113	.113	11 - 10%	Nil.
		M+Am.Chl.	28	.113	.134	.113	.114	-	21 - 18%	Nil.
		M	-	.094	.106	.097	.097	-	12 - 12%	Nil.
J.T.	20	O	-	.112	.110	.112	.110	-	- -	Nil.
		O+Am.Chl.	11	.104	.104	.109	.092	.090	5 - 10%	Nil.
		M+Am.Chl.	36	.123	.134	.117	.100	.081	11 - 10%	Nil.
		M	-	.084	.100	.109	.103	.084	25 - 29%	Nil.
M.McC.	20	O	-	.098	.106	.111	.120	.092	22 - 22%	Nil.
		Special Diet	-	.104	.104	.117	.115	.104	13 - 12%	Nil.
		Sp.D. Am.Chl	9	.119	.122	.137	.125	.-	18 - 15%	Nil.
		O+Am.Chl.	19	.104	.104	.089	.089	.089	- -	Nil.

BLOOD SUGAR CURVES
AFTER
LAEVULOSE

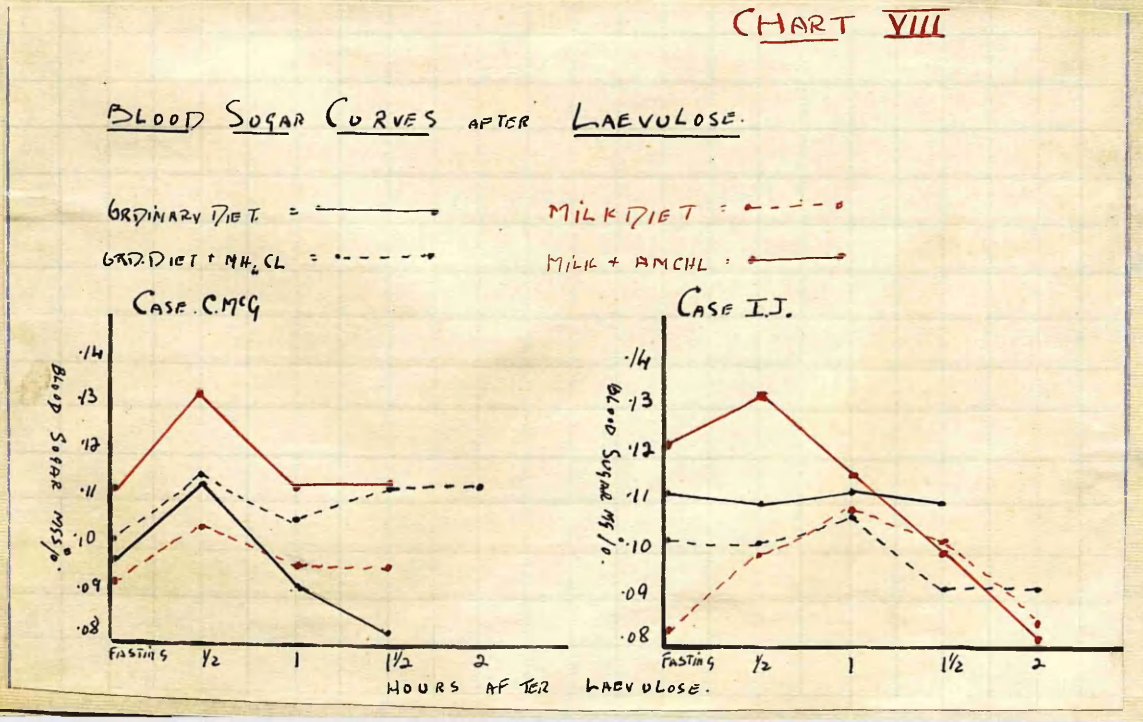


M.McC. was given a diet of ordinary foodstuffs equal in the ratios of Fat, Protein and Carbohydrate to that of a milk diet and the tolerance estimated on this diet and on the administration of the drug. The curves recorded in her case are all within normal limits.

Two of the cases only showed curves at all comparable with those described on a ketogenic diet. In the other cases there is a slight reduction in the tolerance for glucose but none of the curves show the delay in the return to the fasting level which is so characteristic of the "diabetic curve" obtained on a high fat diet.

The test was repeated with laevulose in seven of the cases.

Chart VIII.



In one case only, N.C., was a reduction in the tolerance for laevulose noted.. J.F. showed both on a normal diet and during the administration of Ammonium Chloride a hyperglycaemia of over 30 mgs.% rise but this was equal on both occasions.

Discussion.

These experiments show that the acidosis produced by Ammonium Chloride damages but slightly the ability of the organism to utilise carbohydrate and that it is therefore, not the acidosis per se which is the main factor in the causation of the hyperglycaemia after glucose on a ketogenic diet. The acidosis is well developed by the fourth day yet the blood sugar curve reveals no reduction in tolerance. Lennox (29) found that after 8-20 grm. of Ammonium and Calcium Chloride for 29 days and with a CO_2 of 44 vols.% a subject showed a curve lower than normal whereas after a 17 day fast the blood sugar curve was diabetic in type though acidosis was much less than when receiving Ammonium Chloride. Haldane (73), who showed a marked lowering of glucose tolerance after taking a large dose of Ammonium Chloride, considered that this was due to an interference in the removal of sugar from the blood stream. If this were so one might expect that the hyperglycaemia would be prolonged but this is not shown/

shown in this series of cases.

We do not consider in the light of the above results, that the acidosis is the most important factor producing the disturbance in carbohydrate metabolism.

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SECTION V.

The difference in the acidosis accompanying Ammonium Chloride administration and that produced by a ketogenic diet, is that the latter shows acetonuria. Acetonewas present in varying amounts throughout the period of administration of this diet and Brown and Graham (2) have shown that no matter how small the quantity of acetone present in the urine there is an accompanying acetonaemia. In order to find out if this acetonaemia, or rather, ketosis, played any part in the disturbance in carbohydrate metabolism the following experiments were carried out.

Von Noorden (74) states that glycaemia and glycosuria occur after the administration of acetone and that this condition may be the result of a deficiency in oxygen. Morris and Graham (75), who found that rabbits injected with 2% acetone showed a slight increase in the sugar content of the blood, consider that "this slight rise cannot be attributed to defective oxidation of the blood, although the possibility still remains that acetone inhibits the oxidation processes in the cells". An attempt was made therefore, to give rabbits an "acetonaemia" by injecting acetone into the blood stream. After some days the livers were examined histologically for any change in structure or in glycogen content.

This/

This was carried out in the following manner.

Two rabbits, I and II, weighing respectively .6 and .75 kgrm. were given injections into the ear vein of 20% acetone twice daily for a period of ten days. Rabbit III, used as a control, was given injections of normal saline so that any effect produced by the nervous disturbance occasioned by the injection would be accounted for in this rabbit. The quantity of acetone given equalled for the first four days .8 cc per kgrm. of body weight and was increased on the fifth day to .9 cc per kgrm and on the seventh day to 1 cc per kgrm. The animals were housed under similar conditions and all received the same diet. The urine was tested daily for acetone but there was only occasionally a faint trace registered in the two cases receiving the acetone injections.

After ten days the rabbits were killed by a blow on the back of the head and portions of the liver, kidney and pancreas immediately transferred to Absolute Alcohol and 10% Formalin. These tissues, after going through the various processes required, were stained by Best's Carmine stain for glycogen and by Haemalin and Eosin to demonstrate any structural changes.

No change in any of the tissues of rabbits I and II could/

could be demonstrated, these resembling the tissues of rabbit III in all respects.

Since the quantity of acetone may not have been sufficient to cause any change it was decided to repeat the experiment with larger injections of acetone.

Three rabbits were again taken and rabbit VI weighing 1.1 kgrm. was used as the control animal receiving normal saline injections. Rabbits IV and V, both weighing 1.2 Kilos, were given 20% acetone and the amount given was equal to 1 cc per kgrm. of body weight. The quantity of acetone was increased slightly with each injection and on the evening of the fourth day both were receiving between 2.5 cc and 3 cc of acetone at one injection. On the morning of the fifth day rabbit IV took a slight convulsion after only .5 cc of 20% acetone and became paralysed in the hind quarters. As there was no recovery after 2½ hours the animal was killed by a blow on the head and portions of the liver, kidneys and pancreas transferred at once to 10% Formalin and Absolute Alcohol.

The injections continued however for another six days with rabbit V though the quantity of acetone given thereafter never exceeded 1.5 ccs. twice daily. On the eleventh day rabbits V and VI were killed and treated in a fashion similar to the above.

Figures/

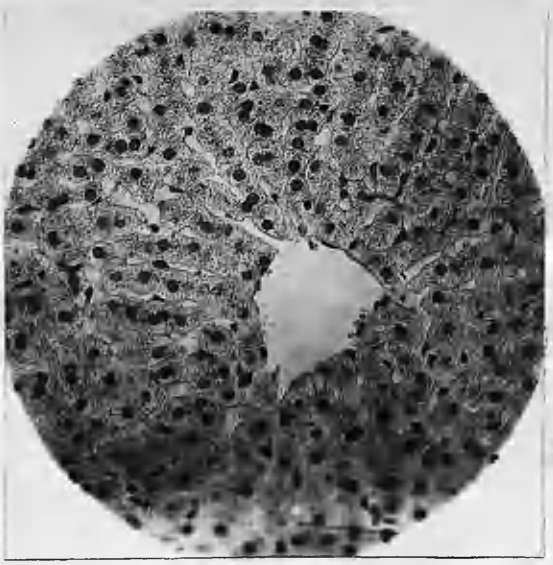

FIGURE 1.

RABBIT IV

STAINED HAEMALUM AND EOSIN

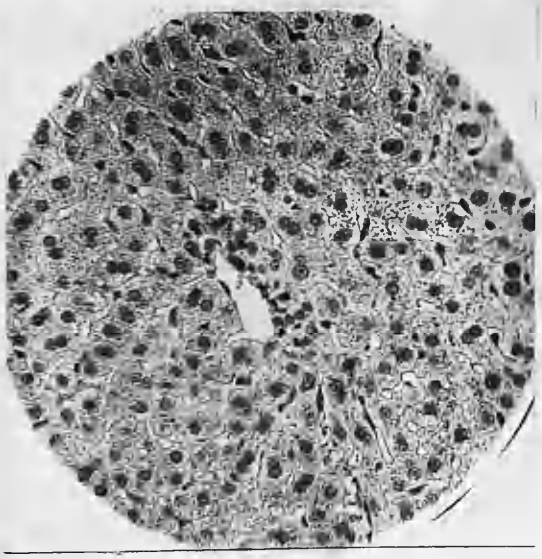

FIGURE 2.

RABBIT V

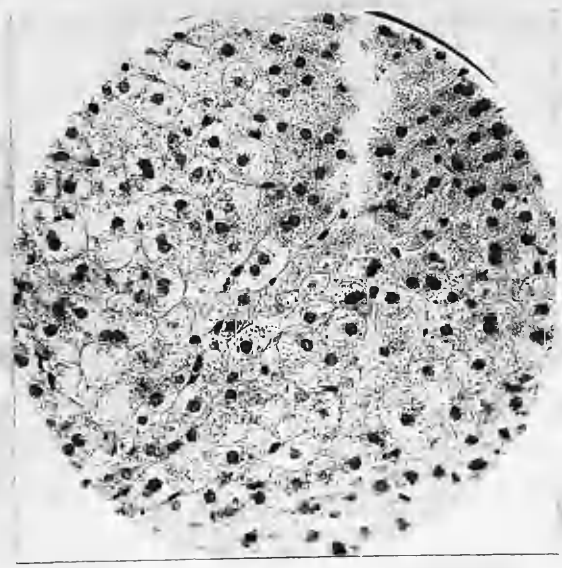

FIGURE 3.

RABBIT VI

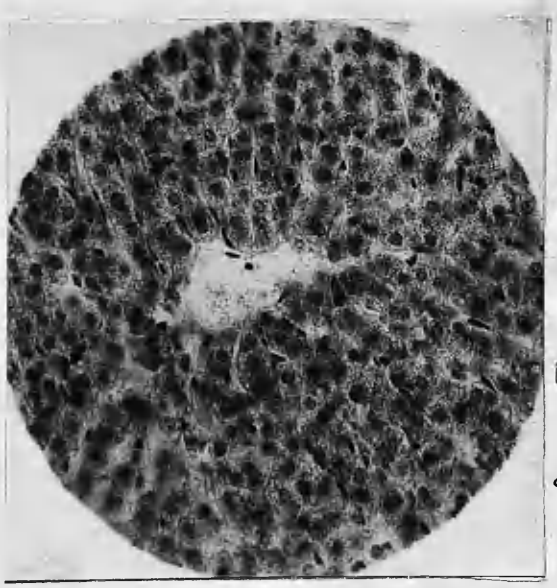

FIGURE 4.

RABBIT IV.

STAINED BEST'S CARMINE.

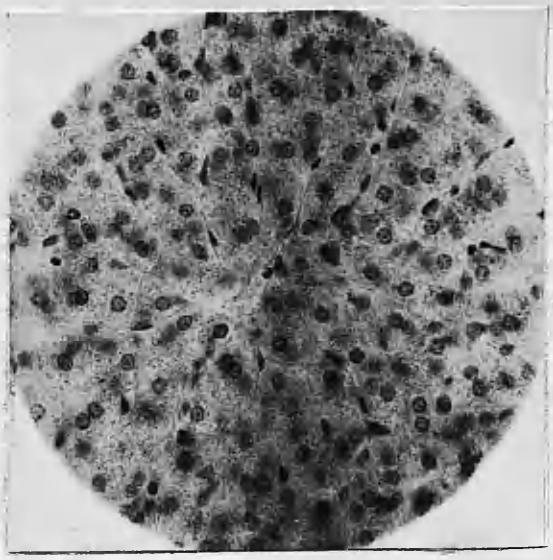

FIGURE 5.

RABBIT V

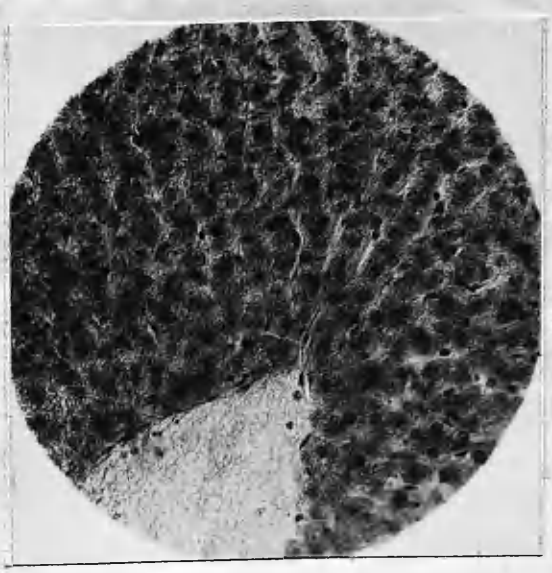

FIGURE 6

RABBIT VI

Figures 1, 2 and 3 are photographs of the livers of rabbits IV, V and VI stained with Haemalum and Eosin. Rabbits IV and V appear normal in structure but that of rabbit VI has empty spaces in the cells showing that some substance, not preserved in the fixing process, has been removed. The cells of rabbit IV do not appear so compact as those of V though they are much more so than those of rabbit VI.

There is a very striking difference in the three slides stained by Best's Carmine Stain. Glycogen shows up as dark granules. If there is an abundance of glycogen the cells are packed with these granules. As can be seen from photographs 4, 5 and 6 there is great variation in the concentration of granules in the cells of rabbits IV, V and VI.

Rabbit IV shows a fair amount of glycogen in the cells around the central canal, there being less or none at all in the cells at the periphery of the lobule. Rabbit VI shows practically all the cells full of glycogen especially around the central canal. Rabbit V presents quite a different appearance, there being practically no granules to be seen, except in a row or two of the cells next to the central canals of the lobules.

Although/

Although further work will be done upon this investigation the effect the acetone seems to have produced upon the liver is very striking. The liver of rabbit V has been emptied of glycogen and rabbit IV who only received acetone for four days shows also some depletion in liver glycogen.

It seems legitimate to conclude therefore that acetone *per se*, with no restriction of diet, produces a depletion of glycogen in the liver and a probable interference with the glycogenic function of the liver.

[I wish to acknowledge here my indebtedness to the Staff of the Institute of Physiology, Glasgow University, and in particular to Mr. Fred Gairns for valuable help in preparing the specimens and slides.]

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Conclusion.

In this thesis it has been shown that there results from the administration of a high fat, low carbohydrate diet a reduction in the ability of the body to deal with glucose. Other workers have demonstrated that after a period of starvation there exists a similar lowering in the tolerance for carbohydrate to that described above and various theories have been put forward to explain this failure in metabolism.

With a high fat diet and during a fast there is a disturbance in acid-base balance, a condition of ketosis and a lack of carbohydrate in the diet.

Du Vigneaud and Karr (28) and Lennox (29) consider that it is the lack of carbohydrate which is the important factor. Maclean and De Wesselow (11) and Foster (76) consider that the factor preventing excessive hyperglycaemia after the ingestion of glucose was glycogen formation and that this factor was stimulated by the hyperglycaemia itself. A second dose of glucose given, therefore, on a falling blood sugar produces no secondary rise since the glycogenic function is already stimulated and able to deal effectively with the glucose. Lennox, (29) bearing this fact in mind, considers it possible that the decreased metabolism of carbohydrate/

carbohydrate has failed to provide the sugar disposing mechanism of the body with the stimulation it needs so that glucose is not quickly utilised and he is inclined to believe that this factor is of more importance than the disturbance in acid-base eqilibrium.

Henderson (77) has pointed out the intimate relationship between acid-base equilibrium and glucose, and Field and Newburgh (78) consider that an increased H ion concentration has a depressing effect on sugar metabolism. Langfeldt, (79) by administering acid, either per se or intravenously, increased the H ion concentration of the blood and produced an increased glycogenolysis. In the present work it has been shown that the acidosis produced by the administration of Ammonium Chloride does not affect the ability of the organism to utilise glucose in a manner at all comparable to the failure experienced while on a ketogenic diet.

Lennox (29) found that the height of the blood sugar, during a fast, increased with the duration of the fast and not with the intensity of the acidosis. We consider that it is not the acidosis which is the factor in the lowering of carbohydrate tolerance. In a case, A.F., of severe clinical acidosis treated in the Royal Hospital for Sick Children the blood sugar (after glucose) was .331% and

the/

the CO_2 of the blood was 30 vols.% at this time. The next day, when the acidosis had gone and the CO_2 had returned to normal, being 72 vols%, the blood sugar was still high being .299% so that the return of the acid-base balance to normal had not influenced the ability of the tissues to utilise glucose. Haldane (73) considers that in Ammonium Chloride acidosis there is a failure to store, but not to oxidise glucose. In the alkalosis produced by the ingestion of Sodium Bicarbonate (accompanying which there is a ketosis) a failure to oxidise glucose arises and consequently a marked lowering in the tolerance for glucose. Corri and Corri (80) have demonstrated that in the summer ketosis of rats there is a diminished capacity to oxidise glucose and a decrease in the glucose tolerance. It may be, therefore, that in Haldane's (73) case it is not the alkalosis which causes the failure to oxidise glucose but the ketosis which accompanies it.

It is this factor of ketosis which seems to be the important one, and the striking depletion in liver glycogen after the injection of acetone shown in Section V of this thesis goes to prove this point. The ketosis interferes with the glycogenic function of the liver. Additional proof that a high fat diet decreases this function of the liver was given by Kaquera. (81) He found that the livers of dogs/

dogs fed on a carbohydrate poor diet formed much less glycogen than those fed on a high carbohydrate diet, when perfused with glucose.

Is the failure in the glycogenic function of the liver due to the ketosis inhibiting the action, or preventing the production of, insulin? Severinghaus (27) considered that this was probably the case. But I have shown that the action of insulin is not inhibited by a ketosis since, on the ketogenic diet, marked hypoglycaemia reactions were elicited by an amount of insulin which on a normal diet produced but a slight response.

I consider therefore that the ketosis inhibits the glycogenic function of the liver and that this is the main factor producing the reduction in tolerance which accompanies the administration of a high fat diet.

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