Effect of High Blood Sugar Levels upon Splanchnic Sugar Output in Experimental Diabetes

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It is known that the elevation of the blood sugar concentration by means of glucose infusion increases the sugar uptake of the peripheral tissues not only in normal but also in pancreatectomized dogs. This was described by Soskin and Levine in 1937 on the basis of their balance studies carried out on eviscerated diabetic dogs.[1] Also in situ measurements (blood flow multiplied by arteriovenous difference) showed that the artificially induced hyperglycemia enhances the sugar uptake of the pancreatectomized dog's muscle three to fourfold (Levine et al.;[2] Issekutz et al.[3]).

On the other hand, little information is available regarding the effect of a sugar load on the hepatic glucose output in diabetes. Whereas in the normal organism, the liver seems to play an important role in reducing the arbitrarily elevated blood sugar level[4-6] it is not clear whether or not this function exists in pancreatectomized dogs lacking insulin. It was of interest, therefore, to investigate the effect of sugar infusion on the hepatic glucose output in the complete absence of functioning islet cells.

Two methods have been employed in these studies:

A. The disappearance rate of blood glucose was followed after a sugar infusion in normal and pancreatectomized dogs without functioning kidneys. The in situ measurement of the amount of glucose taken up by the skeletal muscles allowed an approximate estimation of the splanchnic sugar output or uptake.

B. The hepatic blood flow was measured directly and from the arteriovenous glucose differences the sugar output was calculated. The relationship between the splanchnic sugar output and the blood sugar level was investigated over the range of 60 to 700 mg./100 ml. in normal dogs and of 200 to 800 mg./100 ml. in pancreatectomized dogs.

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METHODS

Group A:

The experiments were carried out on twelve pancreatectomized and six control dogs. Pancreatectomy was performed under aseptic conditions seven to fourteen days before the experiment. The maintenance dosage of Protamine Zinc Insulin was about 0.5 U/kg. once a day. That was withdrawn forty-eight to sixty hours before the experiment. On the day of the experiment, the dogs were given morphine (2 to 3 mg./kg.) subcutaneously, which was followed by an intravenous injection of chloralose (80 to 90 mg./kg.). The kidneys were removed or the ureters were ligated. Consideration of the uniform behavior of the nephrectomized and ureter-ligated animals, in all of the experimental series, indicated that it was not necessary to differentiate between the two groups.

The glucose uptake of muscles was determined by multiplying the blood flow by the arteriovenous sugar difference. Blood flow was measured in the vena profunda femoris by means of our formerly described method.[7] The blood in this vein comes primarily (90 to 95 per cent) from the musculature of the hind leg. After one or two hours' recovery period, the normal values were taken and, following this, the blood sugar level was adjusted by an adequate intravenous priming dose and a subsequent intravenous sugar infusion of two hours' duration, to levels of 366 to 1,105 mg./100 ml. in diabetic animals and to levels of 356 to 953 mg./100 ml. in normal animals. After stopping the infusion, the animal was observed for three to four hours.

If the glucose uptake of the examined muscle area is known, the amount of sugar taken up by the entire body musculature can be computed, assuming that all muscle takes up sugar at the same rate as the examined one. In this calculation, 50 per cent of body weight

was taken for the entire muscle weight of the body.[8] The gross amount of glucose disposed of by the organism can be calculated from the fall of the blood sugar level (mg./100 ml./min.) and the size of the extracellular compartment (25 per cent of body weight). Should this be greater than the amount taken up by the muscles, then the splanchnic area, first of all the liver, must also have taken up sugar. Conversely, if this value is lower than the amount of glucose taken up by the muscle, then glucose must have been released by the splanchnic area, most probably by the liver. The approximate sugar output (+) or uptake (—) of the splanchnic area can be estimated according to the simple equation:

$$\text{splanchnic sugar} \begin{matrix}\text{output}\\ \text{or}\\ \text{uptake}\end{matrix} \text{(mg./min./kg. body weight)} = 5a - 2.5b$$

where "a" represents the glucose uptake of the muscle (mg./100 gm./min.) and "b" is the decrease of the blood sugar (mg./100 ml./min.).*

The first estimation of splanchnic sugar output or uptake, respectively, was done at the last minute of the sugar infusion when a more or less steady state of the blood sugar level was reached. In this case:

$$\text{splanchnic sugar} \begin{matrix}\text{output}\\ \text{or}\\ \text{uptake}\end{matrix} \text{(mg./min./kg.)} = 5a - 2.5b - c$$

where "c" represents the amount of glucose mg./kg./min. infused.

It should be emphasized that this is an approximate gross calculation which does not include the sugar uptake of other tissues (brain, skin, bones). This would increase the value of 5a. The extracellular space could have taken 30 per cent of body weight which would have given a value of 3b instead of 2.5b. However, the observed differences between the pancreatectomized and control dogs, described below, were so striking that the error of this estimation seems not to affect the final conclusion.

Group B:

These investigations were carried out on thirty-two dogs of 12 to 25 kg. body weight. Twenty were pancreatectomized five to seven days previously. Insulin was withdrawn forty-eight hours before the experiment. Twelve dogs served as controls. The ureters were ligated in both pancreatectomized and control dogs to prevent the excretion of sugar and to achieve in the diabetic animals a fairly constant blood sugar level. The rate of the hepatic blood flow was measured as follows: A metal cannula with an attached rubber cuff was introduced into the jugular vein of dogs in morphine-chloralose anesthesia. It was passed through the vena cava superior and inferior until it reached the diaphragm (its position was checked by X ray). Simultaneously a heart catheter, equipped with a thin-walled rubber balloon on its end, was introduced into the left femoral vein. The catheter was passed through the inferior vena cava up to its junction with the hepatic veins. Inflation of the rubber cuff and the balloon with air forced the hepatic blood to flow through the metal cannula into a glass tube filled with physiological saline. This tube contained a glass ball which prevented the mixing of the blood with the saline, which streamed at the rate of the hepatic blood flow into a glass cylinder. The level of the glass cylinder could be adjusted to provide the necessary pressure gradient. The measurement was performed with the help of Anrep's hot-wire anemometer[9] as previously described.[7] After reading the deflection of the galvanometer and taking a sample from the hepatic blood, the normal circulation was re-established by reverting the blood which had been streaming through the cannula. The entire procedure required not more than forty seconds. Blood was taken simultaneously from the right femoral artery.

After determining the splanchnic sugar output or uptake, the blood glucose level was adjusted to 200 to 700 mg./100 ml. in normal dogs and to 300 to 800 mg./100 ml. in diabetic animals, by means of an adequate intravenous priming dose and a subsequent glucose infusion of ninety minutes' duration. The response of the liver was followed for ninety minutes in controls and for two hours in pancreatectomized dogs after stopping the sugar infusion.

*The glucose uptake of 1 kg. muscle: 10a (mg./min./kg.) and that of the entire musculature (50 per cent of body weight: "W"):

$$10a \times 0.5W = 5aW$$

Glucose disappeared from 1 liter of the extracellular space was 10b (mg./min.) and from the entire extracellular space (25 per cent of body weight):

$$10b \times 0.25W = 2.5bW$$

An example:

In a control dog, at the end of the infusion, the sugar uptake of 100 gm. muscle was 2 mg./min. ("a"); the blood sugar decreased in the last thirty minutes of the infusion by 30 mg./100 ml. or 1 mg./100 ml./min. ("b"); the glucose infusion was 20 mg./min./kg. ("c"). Then the splanchnic sugar uptake $5 \times 2 - 2.5 - 20 = -12.5$ mg./min./kg. and sixty minutes after the infusion when the glucose dropped from 440 mg./100 ml. to 140 mg./100 ml. and the sugar uptake of the muscle was found 0.8 mg./100 gm./min. The estimated splanchnic sugar uptake was:

$$5 \times 0.8 - 2.5 \times 5 = -8.5 \text{ mg./min./kg.}$$

The blood glucose was determined according to Somogyi.[10] Statistical calculations were carried out according to Fisher.[11]

RESULTS

Group A:

Figure 1 shows the typical course of an experiment on a normal and on a pancreatectomized dog; both were nephrectomized. In the control dog, the blood sugar level fell quickly when the infusion stopped; in fact, it started to decrease before the end of infusion. Contrary to this, in the pancreatectomized animal the glucose level was steadily rising during the infusion and apart from minor oscillations it did not undergo any significant change during the first three or four hours of observation. The sugar uptake of the left hind leg muscles, at the end of the sugar load, was found to be elevated fivefold and about the same in both control and pancreatectomized dogs. However, at this time, the blood glucose concentrations were 420 mg./100 ml. in the control and 860 mg./100 ml. in the pancreatectomized animal. This seems to confirm the similar finding of Levine et al.[5] After the infusion, the sugar uptake of muscles of the control dog decreased as the blood glucose level fell, whereas in the pancreatectomized animal, the glucose uptake of the left hind leg showed a further increase. The calculated splanchnic response to the sugar load showed a marked difference between the normal and pancreatectomized dogs. Whereas in the normal animal the splanchnic area seemed to respond with a sugar uptake, in the diabetic organism the liver continued to release sugar even at a very high blood sugar level.

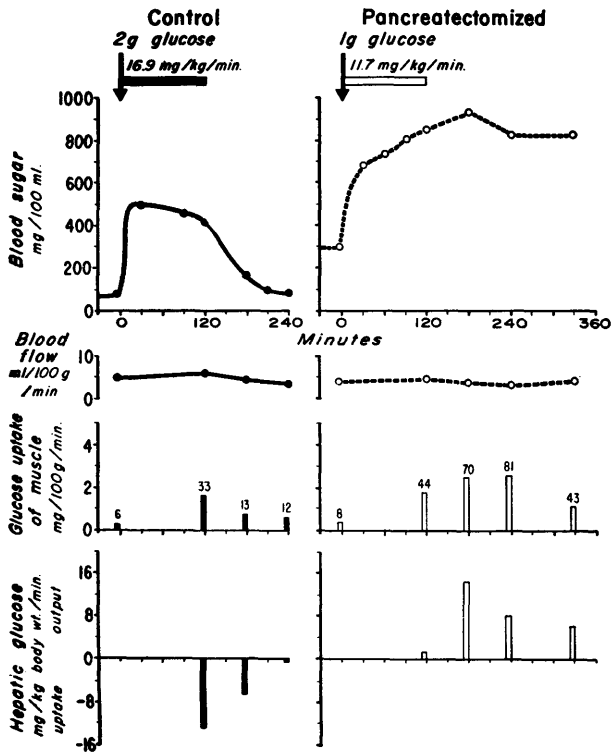

FIG. 1. Effect of sugar load on the peripheral sugar uptake in a control (weight 11 kg.) and in a pancreatectomized dog (weight 12 kg.) (both nephrectomized). Curves and columns from the top downward: blood glucose; blood flow in the muscle; glucose uptake of the muscle (figures above the columns show the arteriovenous sugar difference); approximative splanchnic sugar uptake or output, respectively.

Tables 1 and 2 summarize the results of eighteen similar experiments (twelve pancreatectomized and six controls). They are arranged according to the adjusted blood sugar levels (column 1). Column 3 represents the means (three or four determinations) of the estimated sugar output or uptake of the splanchnic area, *after the end* of infusion during the time intervals in-

TABLE 1

Estimated glucose output of the splanchnic area during hyperglycemia in depancreatized-nephrectomized dogs

Adjusted blood sugar level mg./100 ml.	Duration of observation. Minutes after the end of the glucose infusion	Estimated average glucose output mg./kg. body weight/min.
366	120	+3,5
390	210	—6,8 glucose uptake
443	150	+7,0
637	150	+6,0
638	210	+8,6
739	150	+9,1
865	240	+10,7
865	190	+9,9
870	180	+8,3
906	240	+4,9
1,050	150	+6,2
1,105	240	+12,2

TABLE 2

Estimated glucose uptake of the splanchnic area during hyperglycemia in nephrectomized normal dogs

Adjusted blood sugar level mg./100 ml.	Duration of observation. Minutes after the end of the glucose infusion	Estimated average glucose uptake mg./kg. body weight/min.
396	120	—3,8
417	120	—2,0
447	120	—3,8
507	150	—4,2
511	127	—6,6
953	180	—0,2

dicated by column 2. Nephrectomized diabetic control animals, without sugar load, showed a slight rise of 50 to 80 mg./100 ml. of the blood glucose level in four out of five cases. The fifth dog displayed between the third and fourth hours a sudden increase to above 500 mg./100 ml. blood glucose concentration. The blood sugar level of nephrectomized normal controls was reasonably constant (±5 mg./100 ml.) during the experimental period.

Hematocrit values were measured on sixteen animals, diabetic and control, in which the arbitrarily adjusted blood sugar level varied between 366 and 1,105 mg./100 ml. The mean value of the initial hematocrit was 0.39 ± 0.04. This fell to 0.33 ± 0.04 at the end of the infusion and remained unchanged to the end of the experiment. An average value of 0.33 ± 0.04 was obtained at 150 and 180 minutes, respectively.

Group B:

In continuation and extension of the experiments described under Group A, the splanchnic sugar output or uptake, respectively, was measured directly. Prior to the introduction of the sugar load, the blood glucose level in normal dogs ranged between 65 to 105 mg./100 ml. and in pancreatectomized between 230 and 350 mg./100 ml. In fifty-one observations on twenty normal control dogs (ureters ligated) the average sugar output of the splanchnic area was 6.5 ± 1.3 mg./100 gm. liver/min. or 1.7 ± 0.35 mg./kg. body weight/min. (table 3). The mean hepatic blood flow was 90 ± 7 ml./100 gm. liver/min. Lipscomb and Crandall[12] calculating the hepatic blood flow from the urea excretion found in normal unanesthetized dogs a hepatic glucose output of 2.0 ± 0.2 mg./kg. body weight/min. The glucose output of the splanchnic area seems to depend on the arterial blood sugar level (table 3). The correlation coefficient "r" = 0.640 is significant p <0.001. It should be pointed out, however, that the extremely low sugar output measured at the higher arterial glucose levels (range 90 to 105 mg./100 ml.) was probably caused by an elevated sugar uptake of the intestinal tract. In pancreatectomized animals at an average blood sugar level of 283 mg./100 ml., the splanchnic glucose output was 5.1 ± 1.8 mg./100 gm. liver/min. or 1.9 ± 0.42 mg./kg. body weight/min. which was practically the same found in the controls at the average blood glucose concentration of 84 mg./100 ml. A similar observation was made by Crandall and Lipscomb[13] in pancreatectomized dogs and by Bearn, Billing, and Sherlock[14] in diabetic humans.

A sugar load in the form of an adequate infusion showed a marked difference between normal and pancreatectomized animals. Figure 2 shows two typical experiments. In the normal dog, the arbitrarily elevated blood sugar induced a marked sugar uptake of the splanchnic area. This is essentially the same phenomenon described at first by Soskin and co-workers.[4] No similar effect could be observed in pancreatectomized animals. In spite of the highly elevated blood sugar level (750 mg./100 ml.), the glucose output of the splanchnic area was continued and after the infusion, it seemed

TABLE 3

Splanchnic sugar output in normal dogs

Number of observations	Blood sugar range (mg./100 ml.)	Glucose output mg./min./100 gm. liver	Glucose output mg./min./kg. body weight
19	65-80	14.5	3.34
11	80-90	5.6	1.73
21	90-105	0.8	0.25
Mean±S.E.	84.7±1.9	6.5±1.3	1.72±0.35

Correlation coefficient r = —0.640 p<0.001

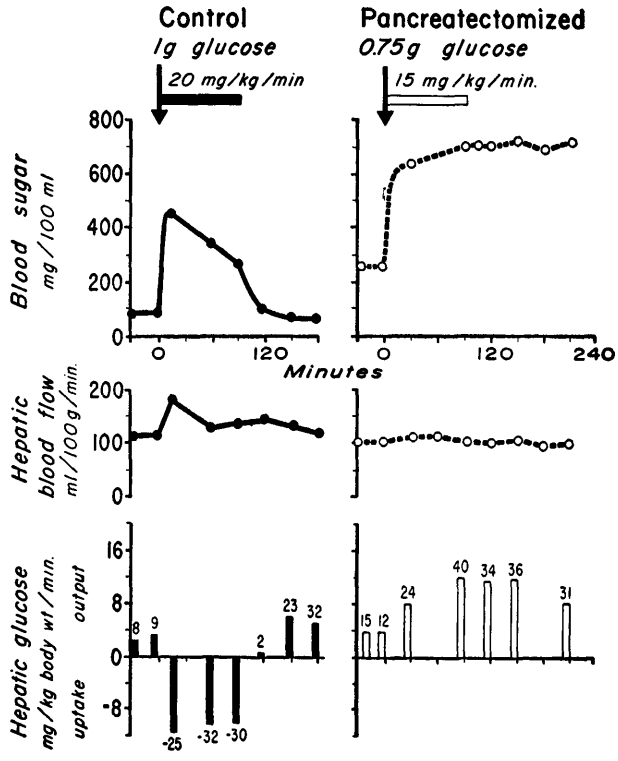

FIG. 2. Effect of sugar load in a control and in a pancreatectomized dog (both ureter ligated). From the top downward: blood sugar; hepatic blood flow (ml./100 gm. liver/min.); splanchnic sugar uptake or output, respectively. Figures above the columns show the hepatic arteriovenous differences.

to be rather increased. Here again as in Group A can be seen a pronounced difference in the rate of the elimination of sugar in the diabetic animal compared with the control dog.

Table 4 summarizes the results obtained at blood glucose concentrations, covering the range between 150 and 700 mg./100 ml. in normal and between 300 and 800 mg./100 ml. in diabetic dogs. The liver of the controls showed "the homeostatic function";[4] that is, a rather high sugar uptake at any level investigated. The deviation from the zero value is significant ($p < 0.01$) in all columns; the maximum is reached between 400 and 500 mg./100 ml. blood glucose. In contrast to this, the pancreatectomized animals did not show any well-pronounced splanchnic uptake of sugar comparable to the controls. In the range between 300 and 500 mg./100 ml., no consistent reaction was observed. In most instances sugar output was obtained but the deviation from the zero was not significant. Greater sugar loads which raised the blood level to 500 or 600 mg./100 ml. caused a marked and significant sugar output. The same is true for the ranges from 600 to 700 mg./100 ml. and from 700 to 800 mg./100 ml. This sugar output is as high or often even higher than that measured at 280 mg./100 ml. level.

DISCUSSION

Two methods have been used to demonstrate the effect of the elevated blood sugar upon the glucose output of the liver of pancreatectomized dogs. Group A, an indirect approach, gave qualitative rather than quantitative information. However, it had the advantage that apart from the ligation of ureters only a small operation (measuring the blood flow in the vena profunda femoris) was needed. The direct determination (Group B) of the hepatic blood flow yielded quantitative data but interfered even if for a short time, with the circulation of the vena cava inferior. It is important to note that both approaches gave essentially the same result, namely, the liver of pancreatectomized dogs put out sugar at arbitrarily elevated blood sugar levels which induced in every control a marked hepatic glucose uptake. Recently, Madison et al.[23] also reported that in pancreatectomized severely diabetic dogs with portacaval shunts, glucose infusion did not produce hepatic sugar uptake. In Group B, at blood sugar levels between 500 and 800 mg./100 ml. (table 3), the average hepatic glucose output of the pancreatectomized dogs was 27.3 mg./100 gm. liver/min. or 8.8 mg./kg. body weight/min. With the indirect method in Group A, at blood glucose concentrations between 443 and 1,105

TABLE 4

Effect of sugar load on the splanchnic glucose output (+) or uptake (—) blood sugar level mg./100 ml.

Range	150-300	300-400	400-500	500-600	600-700	700-800
PANCREATECTOMIZED						
Average blood sugar		351±8	456±7	546±7	640±9	734±12
Splanchnic output mg./min./100 gm. liver		5.2±3.6	9.9±5.5	29.0±8.4	19.6±4.9	37.7±10.9
mg./min./kg. body weight		1.8±1.0	2.8±1.8	8.5±2.1	7.6±2.0	12.1±4.6
t		1.43	1.81	3.45	3.99	3.43
n		15*	14†	15‡	21§	9‡
CONTROLS						
Average blood sugar	216	338	450	631		
Splanchnic uptake mg./min./100 gm. liver	—21.9±6.4	—22.5±6.1	—33.8±7.0	—29.9±4.7		
mg./min./kg. body weight	—5.7±1.8	—5.5±1.7	—9.1±2.1	—8.8±1.6		
t	3.40	3.67	4.79	6.33		
n	11‡	14‡	8‡	5‡		

Indexes mean the significancy of deviation from zero:
n = number of observations (two or three on the same dog)
t = significance of deviation from zero (Fisher)[11]

* $0.1 < p < 0.2$ † $0.05 < p < 0.1$ ‡ $p < 0.01$ § $p < 0.001$

mg./100 ml., the estimated hepatic output averaged 8.2 mg./kg./min.

The mechanism of this paradoxical hepatic response in diabetes needs further investigations. It is probable that the increased activity of adrenal cortex observed in pancreatectomized dogs[15] and in alloxan-diabetic rats[16] was involved in this phenomenon. Also, in humans, it was found that during diabetic coma the urinary excretion of ketosteroids may be considerably increased.[17] It was further shown that hyperglycemia induces an enhanced release of corticosteroids even in nondiabetic animals.[18–20] This may mean an enhanced gluconeogenesis and may explain the finding of Whittlessey and Zubbord[21] that glucose infusion tends to increase the plasma level of ketone bodies in pancreatectomized dogs. Also, Hetenyi and Forbath observed that the glucose load accelerates the increase of the nonprotein nitrogen in the plasma of pancreatectomized dogs.[22]

These observations suggest that a sudden increase of blood sugar (induced for instance by an abundant supply of carbohydrates) may have a "stresslike" effect in severe diabetes. Instead of depressing the hepatic glucose output, the sugar load increases glycolysis and gluconeogenesis, causing a "vicious circle" which may lead to a hyperglycemic coma especially in cases with low insulin sensitivity of the liver.[14]

SUMMARY

The splanchnic glucose output was investigated in pancreatectomized and nephrectomized or ureter-ligated dogs.

An indirect and direct method have been described. In the indirect method, the glucose uptake of the left hind leg muscles was measured in situ. The blood sugar level was adjusted to 366 to 1,105 mg./100 ml. by means of intravenous glucose infusion during a period of 120 minutes. After the infusion, the blood sugar was followed for three to four hours. From the changes of blood sugar level and the glucose uptake of musculature, the splanchnic glucose output was estimated. In a second series of experiments, the hepatic blood flow and the sugar output or uptake of the splanchnic area were directly measured through the right jugular vein. Changes of blood sugar level in the range between 65 and 105 mg./100 ml. showed a correlation with the splanchnic output of glucose in the control dogs. Elevation of the blood sugar levels by means of glucose infusion caused a sugar uptake in controls. No comparable sugar uptake was observed in diabetic dogs, where blood glucose levels above 500 mg./100 ml. increased markedly the splanchnic sugar output.

Both direct and indirect methods brought essentially the same results indicating that the "homeostatic function" of the liver was restricted in diabetes.

SUMMARIO IN INTERLINGUA

Le Effecto de Alte Nivellos de Sucro Sanguinee Super le Rendimento de Sucro Splanchnic in Diabete Experimental

Le rendimento splanchnic de sucro esseva investigate in canes panchrea- e nephrectomisate o uretero-ligate.

Es describite un methodo indirecte e un methodo directe. In le methodo indirecte, le fixation de glucosa per le musculos del gamba sinistro-posterior esseva mesurate in sito. Le nivello del sucro sanguinee esseva adjustate al nivello de 366 a 1.105 mg per 100 ml per medio de un infusion intravenose de glucosa durante un periodo de 120 minutas. Post le infusion, le sucro del sanguine esseva tenite sub observation durante tres a quatro horas. Super le base del alterationes del nivello de sucro sanguinee e le fixation de glucosa in le musculatura, le rendimento splanchnic de glucosa esseva estimate. In un secunde serie de experimentos, le fluxo de sanguine hepatic e le rendimento o le fixation de sucro per le area splanchnic esseva mesurate directemente via le vena dextero-jugular. Alterationes del nivello de sucro sanguinee in le region inter 65 e 105 mg per 100 ml revelava un correlation con le rendimento splanchnic de glucosa in le canes de controlo. Le elevation del nivellos de sucro sanguinee per le infusion de glucosa causava un fixation de sucro in le animales de controlo. Nulle comparabile fixation de sucro esseva observate in canes diabetic in que nivellos de sucro sanguinee de plus que 500 mg per 100 ml resultava in un augmento marcate del rendimento de sucro splanchnic.

Le duo methodos—le directe e le indirecte—produceva essentialmente le mesme resultatos. Isto indica que le "function homeostatic" del hepate es restringite in diabete.

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Diet and Glycoproteins

Although clinical studies of the influence of diet on the carbohydrate-containing proteins of serum (including effects of fasting and feeding of carbohydrate, protein or fat to normal individuals) have proved negative (Z. Stary, *Clin. Chem.* 3:557, 1957), rats have been found to respond to starvation (M. R. Shetlar and C. L. Shetlar, *Proc. Soc. Exp. Biol. & Med.* 88:622, 1955; H. E. Weimer and H. Nishihara, *Ibid.* 95:677, 1957) and protein depletion (H. E. Weimer and H. Nishihara, *loc. cit.*) with a decrease in the serum glycoprotein.

H. E. Weimer and H. Nishihara (*J. Nutrition* 67:137, 1959), have followed up this lead by exploiting the depletion-repletion technic of R. W. Wissler, R. L. Woolridge, C. H. Steffee, Jr., and P. R. Cannon (*J. Immunol.* 52:267, 1946). The animals were fed a protein-free diet until they had lost 25 per cent of their original weight. For repletion the diets contained 10 per cent vegetable oil, 4 per cent salt mixture (U.S.P. XIV) and a vitamin mixture, with the remaining 86 per cent made up of casein and starch. The percentages of protein varied as follows: 0, 8, 17, 27, 40 and 64 per cent.

Total serum glycoprotein, glycoprotein polysaccharide, seromucoid protein and seromucoid polysaccharide were determined. Albumin and globulin proteins were also measured with respect to amounts of protein and polysaccharide. Hematocrits and total hemoglobin were determined.

Hemoglobin and hematocrit values were in the normal range following depletion, but there was a pronounced hemodilution during repletion.

As far as weight repletion was concerned, the 40 per cent protein diet presented the best balance as shown by a more rapid repletion time and a higher food efficiency ratio even when compared to the 64 per cent protein diet.

Protein depletion caused significant reductions in all of the serum glycoproteins with the exception of the polysaccharide component of the albumin fraction. Following repletion on the 8 per cent protein diet, there was some increase in the globulin polysaccharide, but decreases were now observed in the seromucoid and albumin fractions. The 17 per cent protein diet restored total glycoprotein, albumin and globulin polysaccharide values to the normal levels, but there was no effect on the seromucoid fraction, which remained low.

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