THE DIETARY CONTROL OF ALLOXAN DIABETES IN RATS

BY

J. H. BURN, M.D., F.R.S.

Professor of Pharmacology

T. H. C. LEWIS

AND

F. D. KELSEY

(From the Department of Pharmacology, Oxford)

The importance of fat in lessening the severity of diabetes has long been known, but still receives little emphasis. Recently it has been underlined by observations in dogs made diabetic by the daily injection of anterior pituitary extracts. Marks and Young (1939) have found that when these dogs are given a diet which is almost exclusively fat the glycosuria and the ketonuria are greatly diminished. It seems as if on a fat diet the metabolism of these dogs ceases to be abnormal. Recently we have studied the production of diabetes in rats by the injection of alloxan, and have taken the opportunity to observe the effect of dietary changes, in particular of a high-fat diet, upon the glycosuria so produced. Our first experiments showed that the abrupt substitution of a high-fat diet for a normal diet caused a disappearance of glycosuria which was accompanied by some ketonuria. Although this ketonuria was transitory, we endeavoured to avoid it altogether by introducing fat in the diet in stages. In our first attempt to do this we used an unpalatable diet, and the results served only to demonstrate that a reduced calorie intake reduces glycosuria. In other experiments we successfully demonstrated the inhibitory effect of fat on glycosuria when the calorie intake was maintained.

Alloxan has the formula shown, and is closely related to the purines chemically. It is a substance which may arise in the natural course of metabolic processes in the body, and may be one actual cause of diabetes in man.

Shaw Dunn and McLetchie (1943) produced permanent glycosuria in rats by injecting a succession of doses of varying size at varying intervals. Working in April, we observed that two doses given subcutaneously on two successive days, each 16 mg./100 g., produced glycosuria in 8 rats of about 200 g. weight. In two of these the glycosuria disappeared after 4 days, but in 6 rats it persisted. Alloxan seems to be inactive by mouth; for, in 3 rats, four times the above dose on four successive days, followed by eight times the dose on two days, did not cause glycosuria.

Effect of Diet on Glycosuria

In the accompanying Chart is shown the onset of glycosuria in two rats which were kept in the same cage. On the 2nd day, after the injection of the second dose of alloxan, the dextrose excretion rose to 5 g. in 24 hours, and on the 7th day to 7.7 g. The average excretion for the first 13 days was 5.5 g. in 24 hours. During this time the rats received a mixed diet, the composition of which was as follows: 19% ground wheat, 19% bran, 19% oats, 9% ground maize, 9% ground barley, 5% fish meal, 10% meat and bone meal (this being 50% protein), 10% skimmed-milk powder, plus yeast, cod-liver oil, and salt; the calorie value was estimated to be 3.8 cal. per gramme. On the 13th day the diet was changed to a high-fat diet of 90% margarine and 10% casein, plus a salt mixture. As the Chart shows, the glycosuria dropped to 0.5 g. on the first day and disappeared thereafter. In two other pairs of rats the same prompt disappearance of glycosuria was seen on changing from the normal diet to one consisting of 20% casein and 80% margarine. In one pair the dextrose excretion on the normal diet rose during 9 days to 10.9 g. per 24 hours, the mean figure being 7.0 g. On the high-fat diet it fell at once to 0.3 g., 0.2 g., and 0.2 g. on three successive days. On restoration of the normal diet it rose again to a mean figure of 7.6 g. for the next four days. In another pair of rats the normal diet was given for 30 days before it was changed, to observe whether any spontaneous arrest of glycosuria

occurred. The mean dextrose excretion was 3.5 g. daily during this period, and showed no sign of declining. When the diet was changed to a high-fat diet, glycosuria disappeared in the first 24 hours and, save for traces on four occasions, remained absent for 6 weeks, until the rats were again given the normal

Chart showing the daily excretion of dextrose (ordinate) in the urine of three pairs of rats. In the first pair the onset of glycosuria is shown after two injections of alloxan. On the 13th day a high-fat diet was given. In the second pair the effect of 3 days of high-fat diet is shown. In the third pair the effect of high-fat diet is shown after 31 days of normal diet. The high-fat diet abolished the glycosuria in all three pairs.

diet. These observations show clearly that the substitution of a high-fat diet for a normal diet abolishes the glycosuria in rats as found by Marks and Young in the dog.

Fat Diet and Ketonuria

The sudden change to a high-fat diet produced ketonuria in varying degree, as shown in Table I. The ketonuria was

TABLE I.—*Ketonuria on High-fat Diet. (Mg. Acetone per Day.)*

Rats	Fat in Diet	Days of Fat Diet				
		1	2	3	4	5
1st pair ..	90%	2	12	132	49	24
2nd „ ..	80%	2	9	4	—	—
3rd „ ..	80%	0	6	15	0	0

determined by the gravimetric method of van Slyke (1917). Table I shows that the ketonuria in the rats given 90% of fat reached the high figure of 132 mg. on the 3rd day, thereafter declining; it was, however, much less in the two pairs which were given 80% fat.

Gradual Change to Fat Diet.—The rat is an animal which develops ketonuria less readily than man, and, indeed, outside the spring months can eat a diet wholly fat for a week or more with little or no ketonuria (Burn and Ling, 1928). An attempt was therefore made to introduce fat in the diet gradually in order to avoid the occurrence of ketonuria: if ketonuria could not be avoided in the rat it certainly could not be avoided in man.

Importance of Total Calorie Intake

The first experiments were made by changing from the normal diet to a diet of casein, margarine, starch, and a salt mixture. The proportion of casein was kept constant at 20%, while the proportions of carbohydrate and fat were altered; diet of any one composition was continued for a period of not less than 5 days, for which the food consumed was measured and the calorie value calculated. The results in a pair of rats are given in Table II. In the column headed "Diet" the letter S indicates the diet of casein, margarine, and starch, as distinct from N, the normal diet. The figure following S is the percentage of fat in the diet. For each period the mean daily calorie intake and the mean daily dextrose excretion are recorded, and, finally, the body weight of the two rats at the end of each period is shown.

At first, with a diet containing only 40% of fat, the food intake was reasonably good and the dextrose excretion high. On raising the fat percentage to 60 the dextrose excretion dropped very low, but coincidentally with this change the rats ate much less food and the calorie intake fell to little more than half. That the main cause of the diminution of glycosuria was the drop in calories rather than the increased fat was shown by the fact that when the fat percentage was decreased

TABLE II

Diet	No. of Days	Mean Daily Calories	Mean Daily Dextrose Excretion (g.)	Body Weight at End (g.)
S. 40	8	143	3.0	364
S. 60	8	80	0.2	333
S. 50	5	82	0.02	308
S. 40	5	70	0.2	293
S. 30	8	92	0.1	253
N	11	152	2.2	373
N	12	190	5.85	420
N	6	75	0.6	342

in stages to 50, 40, and 30% glycosuria remained very slight. The low calorie intake was reflected in a serious fall in body weight, for at the end of the fifth period the rats had together lost more than 100 g. They were then given the normal diet, 40 g. per day. They ate all of this, and dextrose excretion rose to a mean figure of 2.2 g. In the next period the normal diet was increased, and the calorie intake rose to 190; as a result the dextrose excretion more than doubled. At the end of this period the body weight was fully restored. A final demonstration of the importance of calorie intake on the amount of dextrose excreted in the urine was provided by reducing the diet to 20 g. per day. The dextrose excretion again fell to a low figure.

Effect of Fat on Dextrose Excretion

The attempt to demonstrate the importance of fat had so far failed because the mixture of casein, starch, and margarine was not liked by the rats, and they ate very little. In another pair of rats the experiment was performed by adding fat to the normal diet. The result is shown in Table III. During

TABLE III

Diet	No. of Days	Mean Daily Calories	Mean Daily Dextrose Excretion (g.)	Body Weight at End (g.)
S. 20	6	68	0.3	378
N	6	202	4.9	443
N. 20	6	212	4.0	450
N. 30	6	203	2.8	495
N. 40	6	188	2.3	493
N. 50	10	172	1.0	503
N. 60	16	157	0.3	500
N. 70	6	165	Nil	488

the first period the rats were eating the unpalatable mixture and were then given the normal diet (N). Although the diet was of approximately the same composition the calorie intake rose to about three times the previous value, the body weight increased, and the dextrose excretion rose from 0.3 to 4.9 g. per day. Margarine was then incorporated in the normal diet—first 20%, then 30%, until, finally, 70% was included.

As the percentage of fat rose the mean daily excretion of dextrose steadily fell. With 60% of added margarine the dextrose was as little as 0.3 g. per day, and with 70% the urine became completely sugar-free. While this occurred the calorie intake fell only a little. On the normal diet without added fat the calorie intake was 202 per day at a time when the dextrose excretion was 4.9 g. When the diet contained 70% added fat the calorie intake was 165—that is to say, about 80% of the previous intake—and since 5 g. dextrose, representing 20 calories, was no longer lost in the urine, the calorie usage was 90% of that on the normal diet. The maintenance of the calorie intake was reflected in the maintenance of the body weight, which remained near 490 g. on the diets containing 30, 40, 50, 60, and 70% of added fat. After each change in the diet the urine was examined for acetone bodies on three or more successive days. Ketonuria occurred on only one day of the whole period, which was approximately 8 weeks. It amounted to 19 mg. acetone, and occurred after the rats had

been given 50% of added fat. Strangely enough, there was no ketonuria when the added fat amounted to 60 or 70%. Thus in this experiment glycosuria was abolished by a high-fat diet which did not reduce the calorie intake sufficiently to affect the body weight.

Permanence of the Diabetes

When these diabetic rats were given alternate periods of a normal diet and a high-fat diet it was found that the glycosuria recorded when they were eating the normal diet gradually diminished in successive periods. Figures illustrating this change in one pair of rats are given in Table IV. It is

TABLE IV

Period when Diet Normal	Mean Dextrose Excretion in 24 Hours (g.)
2nd–13th day	5.5
33rd–42nd "	4.4
105th–127th "	2.3
161st–171st "	1.6

The first day was the day on which glycosuria first appeared.

uncertain at present whether this decrease in dextrose excretion was due to intervening periods of high-fat diet or not. Perhaps diabetes produced in rats by alloxan diminishes in course of time even on a normal diet. Further work is in progress to determine whether a high-fat diet not only prevents glycosuria but also restores functional islet tissue in rats.

Discussion

These experiments show how convenient a tool alloxan is for the study of diabetes in groups of animals. Most experimental studies hitherto made have been carried out in dogs or in cats, made diabetic either by removal of the pancreas or by Young's method of repeated injections of anterior pituitary extract, which has the great advantage that the pancreas remains. Even so, the production of diabetes takes a considerable time and requires much anterior-lobe material. Two injections of alloxan are enough to produce diabetes in the rat, so that diabetes can be studied in groups of animals with ease. We have been able to demonstrate that glycosuria is eliminated by diets of high-fat content. Allen (1917) recommended high-fat diets for the treatment of human diabetes, as did Newburgh and Marsh (1920), and Petré (1925) later. Haist, Campbell, and Best (1940) from their studies of the insulin content of the pancreas conclude that fasting, fat-feeding, and the injection of insulin are three factors which allow the pancreatic islets to rest. As already stated, Marks and Young (1939) found that a diet of beef suet diminished both glycosuria and ketonuria in dogs made diabetic by anterior lobe extracts, and Dohan and Lukens (1939) have confirmed this. A diet exclusively fat could not, of course, be given indefinitely. While we have not found it possible to make a sudden transition to a high-fat diet without increasing ketonuria, we have found that by a gradual increase in the fat content of the diet it is possible to abolish glycosuria without producing ketonuria and at the same time to provide a diet containing enough calories to maintain body weight reasonably well. Thus we have been able to demonstrate that alloxan diabetes in the rat can be brought under complete control by diet.

The difficulty in applying these results to man is that most people do not believe that man (in particular, a diabetic patient) can eat a high-fat diet without developing ketonuria and exposing himself to the risk of coma. Our knowledge of the mechanism of ketonuria is still small. Petré (1925) stated that a diet of butter diminished acidosis, whereas a protein diet increased it. Marks and Young (1939) also say: "The results of our investigations have shown that the ketonuria is at a maximum when a meat diet is given, and is lowest with the prolonged feeding of fat." It seems clear that further progress in the dietary control of human diabetes must await a clearer understanding of ketonuria.

Summary

Rats have been made diabetic by the injection of alloxan. When a high-fat diet was substituted for the normal diet the glycosuria disappeared and remained absent until the normal diet was resumed. This abrupt change to a high-fat diet was accompanied by some

ketonuria. When the fat in the diet was increased gradually, glycosuria disappeared without the appearance of ketonuria (except on one day in 8 weeks). The amount of diet eaten containing 70% of added fat provided 90% of the calories available in the amount of normal diet eaten, and maintained body weight.

After successive periods of high-fat diet the glycosuria seen on return to the normal diet steadily diminished.

REFERENCES

- Allen, F. M. (1917). *Amer. J. med. Sci.*, **153**, 313.
 Burn, J. H., and Ling, H. W. (1928). *J. Physiol.*, **65**, 191.
 Dohan, F. C., and Lukens, F. D. W. (1939). *Amer. J. Physiol.*, **125**, 188.
 Dunn, J. Shaw, and McLetchie, N. G. B. (1943). *Lancet*, **2**, 384.
 Haist, R. E., Campbell, J., and Best, C. H. (1940). *New Engl. J. Med.*, **223**, 607.
 Marks, H. P., and Young, F. G. (1939). *J. Endocrinol.*, **1**, 470.
 Newburgh, L. H., and Marsh, P. L. (1920). *Arch. intern. Med.*, **26**, 647.
 Petré, K. (1925). *J. metabol. Res.*, **5**, 7.
 van Slyke, D. D. (1917). *J. biol. Chem.*, **32**, 455.

ANTIBACTERIAL ACTION OF 2-AMINOPHENOL (O-AMINOPHENOL)

BY

MARY BARBER, M.D.

AND

G. A. D. HASLEWOOD, M.Sc., Ph.D.

(From the British Postgraduate Medical School and Guy's Hospital)

In the course of work with a compound between cholic acid and sulphanilamide we discovered that some intermediate substances devoid of sulphanilic groups had a greater activity against Gram-negative bacilli than the final compound. This led to the investigation of a series of compounds for their activity against this group of organisms. Although the investigation is still in progress this paper records the results obtained with 2-aminophenol, as they appear to warrant its trial as a local antiseptic.

The use of sulphonamides and penicillin has brought to light the need for adequate chemotherapeutic treatment of infections due to or kept up by such organisms as *Proteus* and *Ps. pyocyanea*. Acridine derivatives are perhaps the most effective in this respect, although these compounds are toxic to leucocytes and tissue cells. McIntosh and Selbie (1944) recommend a powder consisting of 1 part proflavine and 99 parts sulphathiazole, and claim that it affords protection against pyogenic cocci, clostridia, and Gram-negative bacilli. Recently a group of compounds classified as synthetic detergents have been recommended for local antiseptics. Their action *in vitro* was described by Baker *et al.* (1941), who showed that the cationic detergents had a significant effect on Gram-negative bacilli, although this was less pronounced than their action on Gram-positive organisms. Two of this group—C.T.A.B. and phemeride—have received a clinical trial. The action of C.T.A.B. has been studied by Barnes (1942) and Williams *et al.* (1943, 1944), but unfortunately its antibacterial action is drastically reduced by the presence of organic matter. Phemeride has been shown by Iland (1944) to have an antibacterial action stronger than that of C.T.A.B., to be effective in the presence of 50% blood, and to be relatively non-toxic to the tissues of the body. Unfortunately both these compounds are very much less active against Gram-negative bacilli than against the pyogenic cocci. For the treatment of *Ps. pyocyanea* infections Berry (1944) and Gough *et al.* (1944) have recommended

ethylene glycol monophenyl ether (phenoxetol), which, although a rather feeble antibacterial agent, appears to be particularly active against this organism.

Experimental

In the following experiments proflavine (2 : 8-diaminoacridine sulphate) has been tested in parallel with 2-aminophenol in order that a comparative picture can be obtained. 2-aminophenol is readily soluble in water. Fresh aqueous solutions have a pale-brown colour which deepens on standing, and after a time a slight precipitate forms. In spite of this well-known instability to oxygen and light there is no appreciable difference in bacteriostatic activity between fresh and 7-day-old solutions.

Bacteriostatic Action in Broth.—Serial dilutions were made in broth and then a suitable inoculum of the organism to be tested was added to each tube. Most of the tests were carried out in broth at pH 7.5 containing 1% "lab-lemco," 1% peptone (B.D.H.), 0.5% NaCl, and 0.005% *p*-aminobenzoic acid. Variation of media, however, did not materially affect the results. Table I shows the

TABLE I.—Bacteriostatic Action in Broth at 37° C. (Size of Inoculum less than 500 Organisms in Each Case)

Organism	Limiting Dilution of Chemical causing Complete Inhibition of Growth			
	2-aminophenol		Proflavine	
	1 Day	7 Days	1 Day	7 Days
<i>Bact. typhosum</i> ..	32,000	16,000	64,000	64,000
<i>Proteus vulgaris</i> ..	32,000	32,000	64,000	64,000
<i>Ps. pyocyanea</i> ..	8,000	4,000	8,000	4,000
<i>Staph. aureus</i> ..	64,000	32,000	64,000	64,000
<i>Str. pyogenes</i> ..	64,000	64,000	500,000	500,000

bacteriostatic activity of 2-aminophenol and proflavine obtained with various bacteria when a small inoculum of less than 500 viable organisms was used. Some variation in results occurred in different experiments with 2-aminophenol; the figures given represent an average. The activity of proflavine was rather more constant.

Table II shows the results obtained with *Staph. aureus* when inocula of 3.4 million, 34,000, and 340 viable organisms respectively

TABLE II.—Effect on Antistaphylococcal Action of Varying Size of Inoculum

Size of Inoculum of <i>Staph. aureus</i>	Limiting Dilution of Chemical causing Complete Inhibition of Growth			
	2-aminophenol		Proflavine	
	1 Day	7 Days	1 Day	7 Days
340 viable organisms	128,000	32,000	128,000	64,000
34,000 " "	32,000	32,000	64,000	64,000
3.4 mill. " "	4,000	4,000	64,000	32,000

were used. As will be seen, 2-aminophenol is very much less active against a large inoculum than against a small one, whereas the activity of proflavine is not so greatly affected by the number of organisms.

Bactericidal Tests.—Suspensions of organisms in 2-aminophenol and proflavine respectively were made, such that the final concentration of chemical was 1 : 1000 and the number of organisms per ml. about 20 millions. The tubes were left at room temperature, and viable counts were made after 6 hours and 24 hours. In a typical experiment with *Bact. typhosum* the original concentration of organisms was 17.5 millions per ml. After 6 hours 5.5 millions

TABLE III.—Effect of 2-aminophenol and Proflavine on *Str. pyogenes* in Human Blood in Slide Cells

	Fresh Defibrinated Blood							Blood in 1 : 1000 Liquoid			
	2-aminophenol										
Dilution of chemical:	$\frac{1}{4000}$	$\frac{1}{8000}$	$\frac{1}{16,000}$	$\frac{1}{32,000}$	$\frac{1}{64,000}$	$\frac{1}{128,000}$	None	$\frac{1}{16,000}$	$\frac{1}{32,000}$	$\frac{1}{64,000}$	None
No. of colonies of streptococci ..	0	0	0	0	2	2	2	0	0	32	28
	Proflavine										
Dilution of chemical:	$\frac{1}{100,000}$	$\frac{1}{200,000}$	$\frac{1}{400,000}$	$\frac{1}{800,000}$	$\frac{1}{1.6 \text{ mill.}}$	$\frac{1}{3.2 \text{ mill.}}$	None	$\frac{1}{100,000}$	$\frac{1}{200,000}$	$\frac{1}{400,000}$	None
No. of colonies of streptococci ..	0	0	1	1	2	2	2	0	30	35	28