EXPERIMENTAL STUDIES ON DIABETES.

SERIES I. PRODUCTION AND CONTROL OF DIABETES IN THE DOG. 3. EFFECTS OF PROTEIN DIETS.

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Comparison of Carbohydrate and Protein.

In mildly diabetic dogs the starch of 50 to 100 gm. of bread or cereal is more active in producing glycosuria than the larger quantity of potential carbohydrate represented in a kilo of beef lung.[1] Undoubtedly the starch would be much better assimilated if distributed in small doses over a longer time, but the supposition that the difference is only one of rapidity of absorption and metabolism is opposed by the following facts: (1) The glycosuria from starch in these animals generally lasts over 6 or even 12 hours, while phlorizin and respiration experiments prove that protein requires no longer time for absorption and deamination. (2) The effect of carbohydrate is often cumulative. For example, Dog B2–89 was free from glycosuria on 1 kilo of beef lung daily. On June 25, 50 gm. of bread were substituted for 250 gm. of the lung. The urine remained negative till a glycosuria of 0.75 per cent appeared on June 30 and 0.71 per cent on July 1. Then on return to the diet of 1 kilo of lung, the urine remained negative up to the following test in August. On August 5, 25 gm. of bread were substituted for 150 gm. of lung. A trace of glycosuria appeared on August 10, and 0.18 per cent on August 14. On August 15, nothing was fed but 50 gm. of bread, and an excretion of 1.4 per cent sugar in 140 cc. of urine resulted. On resumption of the diet of 1 kilo of lung glycosuria remained absent up to the time of another experi-

[1] Allen, F. M., *J. Exp. Med.*, 1920, xxxi, 397–399.

ment on September 17. (3) Blood sugar analyses to be reported later show that protein causes little hyperglycemia in mild diabetes, but in severe diabetes it produces a blood sugar curve resembling that of carbohydrate in milder cases, not only in height but also in rapidity of rise, thus confirming the statement (1) above concerning time relations.[2] Also the maximal dextrose-nitrogen ratios in the severest diabetes are a familiar proof that the carbohydrate of protein is excreted quantitatively and is as incapable of assimilation as preformed carbohydrate.

These facts conform to clinical observations and justify the earlier treatment of diabetes, from the time of Rollo onward, inasmuch as the restriction of preformed carbohydrate is after all the foundation stone of dietotherapy, and protein restriction comes secondary in both time and importance.

This simple point also has relation to the question of whether diabetes is a deficiency of utilization of glucose primarily and specifically or of other foods as well. The progressive impairment of protein metabolism, from the stage where protein is apparently assimilated almost perfectly to the stage where it causes hyperglycemia and glycosuria almost identical with those from preformed carbohydrate, has an important bearing upon this question but does not in itself furnish a decisive answer.

Comparisons of Proteins.

Dog B2–29.—This dog, having severe diabetes and a very low tolerance, with a remnant of approximately $\frac{1}{15}$ of the pancreas, was used for feeding tests with approximately 10 gm. of protein in different forms (Table I).

Tests with different forms of protein in other animals gave similar results. Also Dog B2–25, possessing $\frac{1}{12}$–$\frac{1}{15}$ of the pancreas, was used for a more thorough test of pancreas feeding. The body weight was 11.3 kilos on July 4, 1914; 500 gm. of fresh raw beef pancreas were fed daily until July 18, then 600 gm. daily till July 28, then 750 gm. of pancreas till Aug. 2, when the body weight was 14.4 kilos. Slight glycosuria was present from July 30 to Aug. 2. Fasting from Aug. 2 to 10 reduced the body weight to 12.5 kilos. A diet of 500 gm. of beef lung was then given daily to Aug. 18, 600 gm. daily to Aug. 27, 750 gm. daily to Sept. 28,

[2] *Cf.* one example already reported, Allen F. M., *Am. J. Med. Sc.*, 1917, cliii, 362, Chart 8, Dog 386.

800 gm. daily to Oct. 10, 1,000 gm. daily to Oct. 17, 1,200 gm. daily to Oct. 21, and 1,400 gm. daily to Nov. 28. Up to Oct. 29, the lung was boiled after weighing; after that date it was fed raw. Glycosuria was absent till Nov. 25, when 4.1 gm. of sugar were excreted, increasing to 8.2 gm. on Nov. 27. The body weight at this time was 17 kilos. The reason for the higher assimilation of beef lung at a higher body weight was that the dog was gaining tolerance with time. Evidently there is nothing in raw pancreas which alters the natural customary progress of this improvement.

TABLE I.

Dog B2–29.

Date.	Diet.	Glycosuria.
1913		*gm.*
Dec. 29	50 gm. of raw beef.	0
" 31	30 " " soy beans (boiled).	0.2
1914		
Jan. 1	70 " " egg (approximately 2 small eggs), raw.	Trace.
" 2	100 " " lard (no protein).	0
" 3	50 " " raw pancreas.	0.16

Several experiments for comparison of raw and cooked meat (ordinary beef for boiling or beef lung) are typified by the following example.

Dog D4–74.—Female; mongrel; age 4 years; moderately well nourished. Jan. 17, 1917. Partial pancreatectomy leaving remnant estimated at $\frac{1}{11}$. Weight throughout feeding experiments 13.2 to 13.5 kilos. The quantities of beef lung mentioned (Table II) were either boiled after weighing or fed raw.

The literature to which these experiments are related has been reviewed previously.[3] It was confirmed in numerous observations[4] that after a period of carbohydrate-free diet or especially of fasting the feeding of carbohydrate causes glycosuria, which ceases on continuance of the same carbohydrate diet. Apparently some state of unpreparedness of the body for the sudden flood of carbohydrate is

[3] Allen, F. M., Studies concerning glycosuria and diabetes, Cambridge, 1913, 442, 531, 813–815.

[4] *Cf.* Dog B2–63, Mar. 30 and Dec. 16, 1915 (Allen,[1] p. 387); also Paper 4 of this series (*J. Exp. Med.*, 1920, xxxi, 576).

TABLE II.

Dog D4–74.

Date.	Diet.	Glycosuria.
1917		*gm.*
Apr. 16	400 gm. of cooked lung; 100 gm. of suet.	0
" 17	500 " " " " 100 " " "	0
" 18	600 " " " " 100 " " "	0
" 19	600 " " " "	0
" 20	600 " " raw "	0
" 21	700 " " " "	0
" 22	700 " " cooked "	0
" 23	800 " " " "	0
" 24	800 " " " "	0.8
" 25	800 " " raw "	Faint.
" 26	800 " " cooked "	"
" 27	800 " " raw "	0
" 28	900 " " cooked "	Very faint.
" 29	900 " " raw "	0.25
" 30	900 " " cooked "	Faint.
May 1	1,000 " " raw "	Slight.
" 2	1,000 " " cooked "	Faint.
" 3	1,000 " " " "	Very faint.
" 4	1,000 " " " " 25 gm. of bread.	10.9
" 5	1,000 " " raw " 25 " " "	6.7
" 6	1,000 " " " "	5.1
" 7	1,000 " " " "	Slight.
" 8	1,000 " " cooked "	0.5
" 9	1,000 " " raw "	2.2
" 10	1,000 " " cooked "	Faint.
" 11	1,000 " " raw "	1.9
" 12	1,000 " " cooked "	Faint.
" 13	1,000 " " raw "	"
" 14	1,000 " " " "	0
" 15	1,000 " " cooked " 25 gm. of bread.	Faint.
" 16	1,000 " " raw " 25 " " "	3.7
" 17	1,000 " " " "	Faint.
" 18	1,000 " " cooked " 25 gm. of bread.	3.6
" 19	1,000 " " raw " 25 " " "	6.3
" 20	1,000 " " cooked " 25 " " "	0
" 21	1,000 " " raw " 25 " " "	5.7
" 22	1,000 " " cooked " 25 " " "	8.2
" 23	1,000 " " raw " 25 " " "	2.9
" 24	1,000 " " cooked " 25 " " "	3.4
" 25	1,000 " " raw " 25 " " "	4.1
" 26	1,000 " " cooked " 25 " " "	0
" 27	1,000 " " raw " 25 " " "	2.7
" 28	1,000 " " cooked " 25 " " "	2.2
" 29	1,000 " " raw " 25 " " "	1.3
" 30	1,000 " " cooked " 25 " " "	Very faint.
" 31	1,000 " " raw " 25 " " "	Faint.

here represented, just as later authors[5] have proved that a dose of sugar somehow prepares the normal organism so that a second dose is more perfectly assimilated.

Parenteral injection of pancreas extract may lower the sugar in blood or urine, like various other causes of intoxication or prostration, but no therapeutic benefit has ever been found from such treatment.[6] The feeding of pancreas has never benefited diabetes, either in human patients[7] or in dogs. Dogs are of value for these tests because they can eat much more in proportion to the body weight than human patients, but pancreas in either large or small quantity is found to produce glycosuria as readily as any other form of protein. Sandmeyer's observation that, when pancreatic juice is lacking, the improvement of digestion resulting from pancreas feeding may markedly increase glycosuria has been confirmed by Homans,[8] and serves further to discredit the therapeutic usefulness of pancreas or pancreatic preparations given by mouth. Reach's claim that any raw meat may have the same glycosuric influence as pancreas, through some "toxic" action, appears confusing. The necessity of close personal supervision of feeding experiments by the investigator may again be mentioned. Some dogs have a strong repugnance for raw meat; others are ravenous for raw meat and will scarcely touch cooked meat. Fickleness of appetite and digestion is especially to be watched for in the Sandmeyer type of diabetes. The most plausible assumption to explain Reach's results is that his dogs either refused or vomited the cooked meat. The above experiments prove that there is no appreciable difference between cooked and raw meat in regard to either the glycosuria resulting directly from them or their influence on the assimilation of carbohydrate.

[5] Hamman, L., and Hirschman, I. I., *Bull. Johns Hopkins Hosp.*, 1919, xxx, 306.

[6] Allen,[3] pp. 813–819, 855–857. Kleiner, I. S., and Meltzer, S. J., *Proc. Nat. Acad. Sc.*, 1915, i, 338.

[7] Allen, F. M., Stillman, E., and Fitz, R., Total dietary regulation in the treatment of diabetes, Monograph of The Rockefeller Institute for Medical Research, No. 11, New York, 1919, Chapter IV.

[8] Homans, J., *J. Med. Research*, 1915, xxxiii, 1.

Several experiments failed to show any sign of the wide glycosuric differences between proteins alleged by some authors in connection with the oatmeal "cure." There might be some possible interest in exact determinations of blood and urine sugar following the feeding of proteins differing in their content of sugar-forming and non-sugar-forming amino-acids, but the difficulties and uncertainties mentioned with regard to carbohydrate tests are still greater here. The above orientation experiments sufficed to exclude any differences of therapeutic importance.

Results of Immediate Protein Excess.

Dog B2–56.—Female; mongrel; white with brown patch over left eye; age 5 years; good condition; weight 16.5 kilos. Apr. 29, 1914. Removal of pancreatic tissue weighing 24.1 gm. Remnant about main duct estimated at 4.2 gm. (⅛). There was glycosuria for 3 days without feeding, probably because dissection about the pancreas remnant was followed by inflammation in it. Subsequently there was heavy continuous glycosuria on meat diet. May 14. Two tiny bits of tissue, weighing together only a small fraction of a gram, were removed from the pancreas remnant for examination. The remnant at this time did not appear on gross inspection as inflamed. Fasting was then imposed, May 14 to 23, but glycosuria was not reduced below 1.3 per cent and the diabetes was obviously uncontrollable. Meat was then given *ad libitum*, with resulting increase followed by decline of glycosuria and further loss of strength. May 28. The dog weighed 9.7 kilos, was too weak to stand, and was killed for autopsy.

Autopsy.—The pancreas remnant weighed 5.4 gm. and seemed normal in appearance and consistency. The gross autopsy was otherwise negative except for a large abscess in the right axilla, probably derived from a perforating ulcer at the elbow.

Microscopic Examination.—In the tissue removed on May 14 inflammation was limited almost entirely to broad bands of edematous fibrous tissue between lobules, infiltrated with leucocytes and also frequently hemorrhagic, while inside the lobules themselves there was little disturbance. The acini were normal and the islands markedly vacuolated. At autopsy the viscera, including the pancreas, seemed practically normal microscopically. Only slight thickening of trabeculæ remained from the previous infiltration. Acini were normal; islands more extensively vacuolated than before and reduced in size and number.

The diabetes was evidently made more severe by inflammation, but even under these circumstances glycosuria was reduced to traces and was on the point of disappearing with 3 days of fasting following

the first operation. By care in diet at this time control of the diabetes could presumably have been achieved, and after subsidence of the inflammation the recovery of a high tolerance could have been expected. Excessive protein diet removed this possibility and, in conjunction with the inflammation, caused a much more rapid course of diabetes and cachexia than usual.

Results of Prolonged Protein or Protein-Fat Diets.

Examples were previously given[9] of the existence of an apparent limit of protein tolerance in animals with the proper degree of diabetes; and when such a limit is exceeded, downward progress is natural and inevitable. As part of the protein molecule still remains available for nutrition, the decline of weight and strength is generally slower than on high carbohydrate diets, and life generally continues for several months, as illustrated in numerous former experiments. The above record of Dog B2–56 illustrates downward progress on protein as rapid as is usually seen on carbohydrate-rich diets, and also the facts that differences in the course of experimental diabetes are as marked as in clinical diabetes, and that the characteristic changes in the islands are not dependent on preformed carbohydrate in the diet. A less simple problem of protein feeding is presented under the following conditions.

The question arises whether, when an animal has diabetes, as demonstrated by glycosuria on carbohydrate feeding, but is able to eat protein to satiety without glycosuria, or when the protein ration is kept below the apparent tolerance and the full caloric requirements are supplied by addition of fat, diabetes is permanently avoided or merely delayed. It is essential that the test animals should have no changes in the pancreas tending to lower tolerance, and also that the actual permanency of the latent diabetes should be established and a tendency to spontaneous recovery excluded. With these precautions, it may be assumed that animals with simple resection of a portion of the pancreas are free from constitutional or other inherently progressive processes such as may be imagined in human patients. Tests with prolonged protein feeding in them are of the

[9] Allen,[3] p. 588, Dog 38, p. 777, Dog 154.

highest importance, to decide whether (notwithstanding the high content of potential carbohydrate in protein) the difference between protein and carbohydrate diet is absolute, or whether it is a difference of the kind above mentioned between sugar and starch, in that glycosuria is brought on merely more slowly but just as surely. Even with strict quantitative limitation of protein, the question may be stated on a broader basis with regard to the possibility of an impairment affecting the total metabolism in diabetes; namely, whether a diabetic organism can live out its full normal term of life at a full normal level of weight and metabolism by simple limitation of carbohydrate (preformed or from protein) in the diet, or whether the burden of general metabolism will suffice to wear out the weakened function so that an ultimate outbreak of frank diabetic symptoms will result. Such tests upon suitably chosen animals will contribute much toward the question of spontaneous downward progress in human diabetes and the efficacy of the classical treatment based on the idea of restriction of carbohydrate alone. Dogs are specially suited for such experiments, because of their relative insusceptibility to acidosis and other disturbances on pure protein or protein-fat diets.

The first dogs received upon beginning the investigation were set apart for these prolonged experiments. It was inevitable that deaths from distemper, rabies, and other accidents should spoil years of work in some cases, but by starting with a sufficient series of animals and substituting others as needed, some instructive long observations were obtained, of which the following four are the best examples.

Dog B2–00.[10]—Female; mongrel with some bull-terrier blood; brindle; age 3 years; good condition; weight 14 kilos. This dog was received on Oct. 25, 1913, and was subjected to five operations for removal of successive fractions of the pancreas, with periods of many months between so as to allow for any possible compensation by hypertrophy or alteration of assimilation from any cause, and with repeated tests of the carbohydrate tolerance at all stages. Tolerance was maintained for bread and soup with as much as 200 gm. of glucose up to Dec. 16, 1916, when the removal of only 0.1 gm. of pancreatic tissue brought on diabetes, so that bread and soup feeding alone sufficed for slight glycosuria. The dog

[10] See photograph at end of Paper 5 (*J. Exp. Med.*, 1920, xxxi, 587).

passed through two pregnancies in quick succession, the second one terminating on July 16, 1917. The observations up to this point will be described in detail later. Throughout this time she was given 1 kilo of beef lung daily, but was not required to eat all of it; glycosuria was absent except in occasional tolerance tests.

The freedom from glycosuria continuing, the following plasma sugar tests were performed.[11] The time of feeding was between 9 and 10 a.m. daily. July 26. Body weight 13.2 kilos. Dog unwell. Plasma sugar before feeding 0.055 per cent; 2 p.m., 0.099 per cent; 5 p.m., 0.122 per cent; 8.30 p.m., 0.081 per cent. Nov. 27. Weight 14 kilos. 2 p.m. Plasma sugar 0.123 per cent. Dec. 3. Weight 14.5 kilos. Plasma sugar before feeding 0.067 per cent; 5 p.m., 0.108 per cent. Dec. 27. Weight 12.2 kilos. Plasma sugar before feeding 0.128 per cent; 2 p.m., 0.151 per cent; 5 p.m., 0.128 per cent.

TABLE III.

Dog B2-00.

Time.	Aug. 9, 1917.		Oct. 5, 1917.		Nov. 22, 1917.		Dec. 18, 1917.	
	Plasma sugar.	Urine sugar.	Plasma sugar.	Urine sugar.	Plasma sugar.	Urine sugar.	Plasma sugar.	Urine sugar.
	per cent	*gm.*	*per cent*	*gm.*	*per cent*	*gm.*	*per cent*	*gm.*
Before feeding.....	0.081	0	0.109	0	0.109	0	0.141	0
2 hrs. after "	0.164	Very faint.	0.159	0	0.204	Faint.	0.400	3.55
4 " " "	0.109	Faint.	0.145	Faint.	0.238	0.14	0.384	2.10
6 " " "	0.135	"	0.152	0.14	0.125	0.11	0.357	3.95

Four tolerance tests also indicated a decline of tolerance during the above period of high protein diet. These tests consisted in giving, on the 4 days mentioned, an identical test diet of 200 gm. of bread, 150 gm. of glucose, and 100 gm. of beef lung, and determining the blood and urine sugars at the intervals shown in Table III.

Jan. 2, 1918. Diet changed to 400 gm. of lung and 50 gm. of suet. Jan. 15. Weight 12 kilos. Plasma sugar before feeding 0.139 per cent; 5 p.m., 0.161 per cent. Jan. 17. Diet changed to bones only. Jan. 21, 2 p.m. Plasma sugar 0.164 per cent. Jan. 28. Weight 11.1 kilos. Plasma sugar before feeding 0.133 per cent. Diet changed to 100 gm. of lung and 100 gm. of suet. Feb. 6, 2 p.m. Plasma sugar 0.155 per cent. Feb. 11. Plasma sugar before feeding 0.112 per cent; 2 p.m., 0.123 per cent. Feb. 14. Diet increased to 300 gm. of lung and 100 gm. of suet. Mar. 14. Diet increased to 500 gm. of lung and 100 gm. of suet.

[11] The methods of Lewis and Benedict (Lewis, R. C., and Benedict, S. R., *J. Biol. Chem.*, 1915, xx, 61), and of Benedict (Benedict, S. R., *J. Biol. Chem.*, 1918, xxxiv, 203) have been used for all blood sugar analyses.

On protein diet from July 26, 1917, to January 15, 1918, the dog showed downward progress as evidenced by the increasing hyperglycemia and the tolerance tests. To exclude the possibility that such a hyperglycemic tendency might be due to something inherent in the dog, undernutrition was instituted on January 17 with a diet of nothing but fresh bones, and thereafter the quantity of protein was restricted, the maximum of 500 gm. of lung being attained by March 14. Hyperglycemia which is slow in onset, due to prolonged excess in protein or fat, is also slow in subsiding; but with the decline in body weight the blood sugar came gradually to the normal level. The last mentioned diet and the freedom from glycosuria continued to August, 1918.

Dog B2–01.[10]—Female; bull and fox-terrier mongrel; white with brown markings; age 2 years; good condition; weight 14 kilos. Received Oct. 25, 1913, and used at first like Dog B2–00 for removal of successive portions of pancreas.[12] The removal of 0.8 gm. of tissue in the final operation on Aug. 31, 1916, made the animal potentially diabetic.[13] The tolerance was at first so high that bread diet with 300 gm. of glucose caused no glycosuria. It declined till glycosuria resulted from bread alone, then fluctuated according to the body weight, but on the whole fell in consequence of prolonged slight overfeeding, so that by June 30, 1917, there was hyperglycemia when the dog was fat on a diet of 400 gm. of lung and 200 gm. of suet. The weight was kept low and the tolerance high during the remainder of that year. In 1918 the dog was allowed to gain weight, on a diet which after Feb. 13 was 400 gm. of lung and 100 gm. of suet. The plasma sugar was normal (0.089 per cent) before feeding in the last analysis on Feb. 4.

Dog D4–52.[10]—Female; mongrel; yellow and white; age 3 years; good condition; weight 12 kilos. Nov. 24, 1916. Received with pups, which she reared. June 27, 1917. Removal of pancreatic tissue weighing 22.9 gm.; remnant about main duct estimated at 3.4 gm. ($\frac{1}{8}$). Glycosuria at first was heavy on feeding of bread and soup with glucose up to 200 gm., but soon ceased. July 12. 0.35 gm. of additional pancreatic tissue was removed, and the same experience with feeding was repeated. July 20. 0.2 gm. of additional pancreatic tissue was removed, and again the glycosuria from bread and 200 gm. of glucose was transitory. Aug. 3. A final fragment of 0.3 gm. of pancreatic tissue was removed. Glycosuria was absent on bread diet until Aug. 8, when the addition of 200 gm. of glucose made it heavy. On this glucose mixture the sugar excretion gradually diminished and ceased on Aug. 13.

[12] These experiments will be described later.

[13] The subsequent feeding tests will be described later.

After Aug. 21, glucose was omitted and the diet was plain bread and soup. Slight glycosuria appeared on Oct. 10, and the diet was therefore changed to 500 gm. of beef lung and 100 gm. of suet. The same diet was continued regularly, though frequently the dog left considerable of it uneaten. By June, 1918, the weight had risen to a maximum of 12.6 kilos. Tests were performed at intervals (Table IV).

TABLE IV.

Dog D4–52.

Feeding of 500 gm. of lung, 100 gm. of suet, and 100 gm. of bread.

Date.	Weight.	Plasma sugar.			Urine volume.	Glycosuria.
		Before feeding.	3 hrs. after feeding.	6 hrs. after feeding.		
1917	*kg.*	*per cent*	*per cent*	*per cent*	*cc.*	*per cent*
Mar. 1	11.7	0.110	0.122	0.109	420	0
Oct. 25	11.6				580	2.44
Nov. 14	11.7	0.092	0.294	0.246	410	1.1

TABLE V.

Dog D4–52.

Feeding of 300 gm. of bread and 200 gm. of glucose.

Date.	Weight.	Plasma sugar.				Glycosuria (24 hrs.).
		Before feeding.	2½ hrs. after feeding.	4 hrs. after feeding.	6½ hrs. after feeding.	
1917	*kg.*	*per cent*	*per cent*	*per cent*	*per cent*	*gm.*
Aug. 8	11.5	0.149		0.312	0.092	6.75
" 15	11.6	0.101		0.172	0.161	0
Oct. 5	11.5	0.208	0.334	0.384	0.416	18.0

The tests mentioned above merely compare the diabetic condition on October 25 and November 14 with the normal state on the preceding March 1. As far as can be judged from glycosuria, there was gain rather than loss of tolerance from October 25 to November 14, though such differences may be accidental.

From the tests in Table V, it is seen that on August 8 (5 days after the last pancreas operation) the above glucose mixture caused hyperglycemia and glycosuria, which were brief, both being ended within the $6\frac{1}{2}$ hour experimental period. By August 15, the bread and glucose

diet having been continued in the interval, tolerance had apparently been gained, so that glycosuria no longer resulted; but the curve of hyperglycemia, though lower, was longer. Delayed absorption is one possible factor here. The dog tired of glucose before diabetes was produced, so that after August it had to be discontinued, as already stated. On plain bread diet thereafter glycosuria was absent, but hyperglycemia was evidently present and tolerance was lost markedly. This was shown in the tolerance test of October 5, in which the blood sugar was high at the outset and ran a prolonged high course. Of the 18 gm. output, only 7.9 gm. were excreted during the $6\frac{1}{2}$ hour period, and the rest over night. A fast day was given on October 6, to allow recovery from the test. Nevertheless, glycosuria on plain bread and soup feeding began on October 10 as stated. This experiment is another illustration of downward progress on starch diet, and also of downward progress with hyperglycemia without glycosuria. A recuperative effort on the part of the pancreas remnant is manifest, but it was overcome by the excessive feeding.

Intravenous Glucose Tests.

The intravenous injections were given discontinuously, in a manner described in detail in a later paper. The dosage was constant, on the assumed normal weight of 12 kilos, without regard to the changes in actual weight. This meant the injection of 30 cc. of 10 per cent glucose solution every 15 minutes, in order to give 1 gm. per kilo per hour (Table VI). Feeding was omitted on each injection day and the day following. Excitement and other known causes of disturbance were avoided.

The experiment on August 6, 3 days after the last pancreas operation, seems to show the same characteristics as the above described feeding test on August 8; namely, beginning with an existing hyperglycemia, the blood and urine sugars rose quickly to high levels, but tended to fall toward the close, the relatively low percentages of the 7th and 8th hours being particularly striking.

The observations of November 19 and February 19 show the existence of hyperglycemia on prolonged carbohydrate-free diet, and downward progress of the diabetes notwithstanding absence of glycosuria.

TABLE VI.

Dog D4–52.

Intravenous glucose injections, 1 gm. per kilo per hour in 10 per cent solution (four injections per hour).

Time.	Plasma sugar.	Urine.	
		Volume.	Glucose.
Aug. 6, 1917. Weight 10.75 kilos.			
	per cent	*cc.*	*per cent*
Before injection	0.218		0
At end of 1st hr.	0.500	16	2.64
" " " 2nd "	0.715	16	5.08
" " " 3rd "	0.475	54	2.84
" " " 4th "	0.590	52	1.54
" " " 5th "	0.415	76	0.74
" " " 6th "	0.270	78	1.81
" " " 7th "	0.202	94	1.45
" " " 8th "	0.125	82	0.82
1 hr. after injection	0.128	53	0.19
2 hrs. " "	0.130	12	Faint.
3 " " "	0.133	29	0
Glucose excreted			7.8 gm.
Nov. 19, 1917. Weight 11.7 kilos.			
Before injection	0.169		0
At end of 1st hr.	0.555	36	1.93
" " " 2nd "	0.555	68	3.08
" " " 3rd "	0.435	82	1.49
" " " 4th "	0.370	132	0.79
" " " 5th "	0.370	98	0.39
" " " 6th "	0.356	126	0.28
" " " 7th "	0.370	127	0.55
" " " 8th "	0.370	105	0.39
" " " 9th "	0.384	105	0.36
" " " 10th "	0.322	101	0.42
1 hr. after injection	0.156	45	Faint.
2 hrs. " "	0.147	10	0
Glucose excreted			7.7 gm.
Feb. 19, 1918. Weight 12 kilos.			
Before injection	0.145		0
At end of 1st hr.	0.417	20	4.77
" " " 2nd "	0.476	55	5.13
" " " 3rd "	0.500	114	3.39
" " " 4th "	0.525	109	3.45
" " " 5th "	0.500	102	3.40
" " " 6th "	0.475	140	2.78
" " " 7th "	0.455	90	4.35
" " " 8th "	0.384	87	3.23
1 hr. after injection	0.294	43	0.74
2 hrs. " "	0.170	19	Very faint.
Glucose excreted			25.7 gm.

Dog D4–69.—Male; black collie mongrel; age 3 years; good condition; weight 15.5 kilos. Jan. 5, 1917. Received. The removal of a kidney on this date was presumably without effect on the production of diabetes. Successive portions of pancreatic tissue were removed on Jan. 23, Feb. 23, Mar. 28, Apr. 19, May 8, June 1, June 27, and July 20. Tests of the tolerance were made between all the above operations, and diets of bread and soup with as high as 400 gm. of glucose, though causing more and more glycosuria, failed to maintain it permanently. After the last operation, which involved the removal of only 0.5 gm. of tissue, glycosuria was absent on bread and soup diet, but heavy at first with the addition of 100 gm. of glucose. As it tended to diminish, the glucose was increased on Aug. 8 to 200 gm., but again the heavy glycosuria diminished, became intermittent, and ceased. No glycosuria occurred after Aug. 15. Aug. 21. Glucose

TABLE VII.

Dog D4–69.

Feeding of bread and soup and 200 gm. of glucose.

Date.	Plasma sugar.			Glycosuria (24 hrs.).
	Before feeding.	3½ hrs. after feeding.	5½ hrs. after feeding.	
1917	*per cent*	*per cent*	*per cent*	*gm.*
Aug. 8	0.133	0.344	0.310	4.5
" 9	0.116	0.238	0.264	Trace.
" 15	0.110	0.217	0.212	0
Oct. 5	0.200	0.525	0.435	24.8

was discontinued, and the diet thereafter was plain bread and soup. The weight had risen by Oct. 5 to 17.6 kilos. Tolerance tests were performed during this period (Table VII).

Though 2 fast days were imposed following Oct. 5, the former sugar freedom on bread diet was no longer possible, the glycosuria in repeated attempts being persistent and increasing. Therefore, beginning Oct. 17, the diet was 100 gm. of suet and quantities of lung varying from 400 to 1,000 gm. Oct. 25 to 30. A diet of 500 gm. of lung and 100 to 150 gm. of bread caused continuous glycosuria. After this the diet was 500 gm. of lung and 100 gm. of suet, with continuous absence of glycosuria except on a few test days.

Nov. 14. Addition of 100 gm. of bread to the diet caused glycosuria of 0.3 per cent in 350 cc. of 24 hour urine. The plasma sugar before feeding was 0.127 per cent; 3 hours after feeding, 0.244 per cent; 6 hours after feeding, 0.138 per cent.

Intravenous tolerance tests were performed as shown in Table VIII.

Experiments like those on Dogs B2–00, B2–01, D4–52, and D4–69, with their requirements of prolonged care in diets, daily urinalyses, and attention to numerous details, are full of difficulties. These four dogs were the survivors of a series in which the attempt was made to reproduce the conditions of human patients as closely as possible. The above records extend to the summer of 1918, when the writer entered military service, and the dogs were left on the

TABLE VIII.

Dog D4–69.

Intravenous injection of 1.3 gm. per kilo per hour of a 10 per cent solution of glucose.

Time.	Aug. 6, 1917. (Weight 16.2 kilos.)			Dec. 4, 1917. (Weight 14.2 kilos.)			Feb. 19, 1918. (Weight 15.5 kilos.)		
	Plasma sugar.	Urine.		Plasma sugar.	Urine.		Plasma sugar.	Urine.	
		Volume.	Glucose.		Volume.	Glucose.		Volume.	Glucose.
	per cent	*cc.*	*per cent*	*per cent*	*cc.*	*per cent*	*per cent*	*cc.*	*per cent*
Before injection.	0.106	10	0	0.104		0	0.154	32	0
After 1st hr.	0.278	20	1.56	0.715	100	2.84	0.625	46	4.83
" 2nd "	0.294	30	2.87	0.990	144	4.45	0.800	214	4.00
" 3rd "	0.312	64	1.71	0.990	222	3.65	1.000	252	4.35
" 4th "	0.270	106	0.76	1.000	300	2.80	1.180	274	3.70
" 5th "	0.218	110	0.42	0.844	220	2.93	1.110	281	4.25
" 6th "	0.204	76	0.53	0.844	265	2.43	1.050	270	4.55
" 7th "	0.183	128	0.51	0.625	222	2.71	1.000	222	4.55
" 8th "				0.715	216	3.82	0.950	278	5.13
1 hr. after last injection.	0.082	90	Trace.	0.370	60	3.60	0.525	78	5.88
2 hrs. " " "	0.098	82	0	0.217	66	0.49	0.322	10	5.13
Glucose excreted.....			4.5 gm.			55.2 gm.			80.4 gm.

Both the feeding and the intravenous tests indicated gradual loss of tolerance in the absence of glycosuria, first on starch and later on protein-fat diet. The sugar curves of Feb. 19, 1918, were not only the highest and most prolonged, but also with the initial figure of 0.154 per cent indicated that hyperglycemia was now constant on the regular diet of lung and suet. In the final blood taken at the height of digestion at 2.30 p.m. on Mar. 20, 1918, the plasma sugar was 0.256 per cent, still without glycosuria. The weight was gradually falling, and was 14.75 kilos at this time.

diets stated under the care of the animal attendants. They were seen once during the winter, sufficiently to learn that all four were free from glycosuria; three of them were vigorous and fat, but Dog D4–69 was thin and in rather poor condition. In the late summer of 1919, two of the dogs were found to have hyperglycemia and glycosuria, and were therefore transferred from The Rockefeller Institute to the writer's clinic. The two which were sugar-free were cared for at the farm of the Institute in New Jersey.

The animal in worst condition was Dog D4–69, which was thin, with hopeless hyperglycemia and glycosuria, and which died of diabetes on October 10, 1919. Dog B2–01 was strong and fat at a weight of 14.8 kilos, but showed heavy sugar and acetone reactions. Glycosuria was at first abolished by fasting, but returned, owing to persistence of hyperglycemia when attempts were made to feed. Rigorous undernutrition at this time might have proved successful, but the mistaken laxness, due to the deceptive strength and fatness of the animal, soon ended in a hopeless condition, which was uncontrollable by fasting, so that death occurred November 15, 1919. The record of the terminal period is given in the next paper. The other two dogs were in excellent condition when visited at the farm on November 3, 1919. Plasma sugar analyses on that day were as follows: Dog B2–00, before feeding, 0.092 per cent; 4 hours after feeding, 0.136 per cent; Dog D4–52, before feeding, 0.093 per cent; 4 hours after feeding, 0.123 per cent.

It will be seen that the experiment consisted in taking four dogs with different grades of diabetes, which had been produced by sufficiently long experimental procedures to rule out accidental influences as far as possible, and had been tested in various feeding and injection experiments, and then placing them on nearly identical diets on which they were free from glycosuria for extended periods. The ultimate outcome could in the main have been predicted from the preliminary observations.

Dog D4–69 had continuous hyperglycemia in the spring of 1918, and in the absence of extraneous interfering factors such a condition in dogs always leads to manifest and fatal diabetes. Loss of weight may have been due to the latent diabetes or to indigestion or other causes; it evidently postponed the outbreak of active dia-

betes longer than usual. Dog B2–01 exhibited hyperglycemia and lowered tolerance when obese in the summer of 1917, and it was therefore to be expected that a similar result would follow when she became fat on the same protein ration in 1918–19. These records illustrate that in dogs, as in human patients, a true recuperative power is limited to the earlier stages of diabetes, and when diabetes has lasted many months or years the assimilation may undergo apparent fluctuations according to the diet and weight but is not capable of any great restoration in an absolute sense. The final purpose was, after the expected onset of diabetes, to check this as before by fasting and reduced diet, and then prove that the animals could be kept indefinitely in the undernourished condition free from diabetes or downward progress, on the same protein ration; in other words, to show the influence of dietary fat and body weight in causing downward progress. This fact was established by other experiments,[12] but the opportunity of testing the permanency of such arrest of diabetes and the feasibility of protecting the assimilation by undernutrition extending over many years or the full lifetime of the dogs was lost.

Dogs B2–00 and D4–52 had milder diabetes. Both showed downward progress on excessive diets of carbohydrate or protein, and both illustrated the fact, often observed in human patients, that when mild diabetes is checked by a diet within the actual tolerance, hyperglycemia may persist for a long time but gradually subsides. The question was what would happen to such animals if they were allowed to live indefinitely on a diet which seemed to be within the tolerance. The result could not be predicted in advance, and the outcome to date establishes the following conclusions.

1. No inherent downward progress is perceptible. Dog B2–00 has been kept for 6 years, and has been demonstrably diabetic for 3 years. The downward progress observed in other animals, and also in these animals on excessive diets, is purely the result of food injury; in other words, to functional overstimulation of the pancreas as an endocrine organ.

2. The benefit of the classical treatment of diabetes is confirmed. With the susceptibility of both these animals to injury from excess of either carbohydrate or protein demonstrated, it is evident that

this injury was checked when carbohydrate was omitted, protein restricted, and a full caloric diet made up by the use of fat. Fat is evidently less injurious than carbohydrate or protein, and its harmfulness is chiefly noticeable in the more severe grades of diabetes. It is safer for these animals to be obese on a fat diet than to eat carbohydrate or a carbohydrate-forming food such as protein. By inference, fat is not a direct source of carbohydrate.

3. The future outcome in these two animals holds several possibilities. (*a*) One question concerns the degree and permanence of their assimilative power. Other experiments justify the assumption that by undernutrition their tolerance could be greatly raised, so that they might take considerable protein and carbohydrate, and by increased obesity the tolerance could be further lowered, probably to the point of glycosuria on their present protein ration. The question is whether their tolerance at their present weight is high enough for permanent assimilation of the limited carbohydrate derivable from their present diet; whether the difference between fat and protein is absolute or merely a matter of time, like the differences between glucose and starch or between starch and protein; and therefore whether diabetes is primarily a deficiency of the total metabolism or of carbohydrate metabolism alone. (*b*) The plasma sugars under present conditions are not absolutely normal. Particularly in Dog B2–00 the figure of 0.136 per cent during digestion of protein is above normal, but yet is within the limits permitted in numerous diabetic patients. There is a question whether this slight and temporary overload of each day can be borne indefinitely by the pancreas, or whether it is the first small sign of a breaking strain. (*c*) In addition to the simple prolonged functional wear and tear, other influences such as age enter in, and it is of interest to know whether senility will bring an onset of diabetes, as it seems to do in so many human patients. If necessary, the direction of progress in the animals can be judged at any time by tolerance tests, which were instituted in the first place in order to permit of such comparisons, but it seems better to avoid this source of possible injury. As these dogs bear such a close resemblance to mildly diabetic patients kept sugar-free by restriction only of sources of carbohydrate, it seems most valuable to learn whether they can remain fat and lazy indefinitely

with impunity, or whether even the mildest diabetes will ultimately undergo aggravation from luxus diets.[14]

CONCLUSIONS.

No specific differences were observed between the glycosuric effects of different kinds of protein. Other incidental observations were noted in connection with the records of individual animals. General conclusions are deferred to the close of the series.

[14] Both these dogs recently died and were autopsied. Dog D4-52 had a large calculus in the bladder but otherwise appeared to be in excellent condition. No cause of death was found. Dog B2-00 was more closely observed; there was a history of refusal of food, and drinking and vomiting of water. The urine in the bladder was heavy with sugar; the subcutaneous tissue and peritoneum contained abundant fat, and the liver was intensely fatty. It is therefore certain that the death of this animal was due to acidosis. The same possibility exists for Dog D4-52 but is unproved. The results indicate that luxus diets lead to a fatal termination in diabetes.