



Biological Study of Fifty-Six-Day Protein-Free Diet in Dogs

DONALD DAWSON, M.D.* AND J. GARROTT ALLEN, M.D.†

THIS biological study was designed to ascertain various physiologic and histopathologic changes in dogs given a basal diet of no protein but only a prepared calorie mix computed at 100 cal. per kg.¹⁻³

The constituents of this calorie diet are standard in this laboratory and have been found to be palatable for dogs. In our own experience, this total number of calories per kilogram has been found to be an entirely adequate caloric intake in various investigations in which dogs have been the test animals used. The composition of the mixture is as follows:

Sucrose	2,720	gm.
Dextrin	280	gm.
Agar	40	gm.
Wesson's salt	160	gm.
Choline	12	gm.
Paba	0.6	gm.
Lard	600	gm.
Mazola	200	gm.

The final product comes to approximately 480 cal./100 gm.

DESIGN AND METHOD

Our aim was to give this preparation as the sole feeding for a period of fifty-six days, noting the gross biological and histopathologic changes during and at the end of the test period. At the termination of the fifty-six-day test period,

the following surgical procedures were performed under light nembutal anesthesia: left nephrectomy and adrenalectomy, with a wedge resection of liver and partial pancreatectomy. The tissues were stained with hematoxylin and eosin and Sudan III stains. A total of twenty healthy mongrel dogs that were known to be free of parasitic gastrointestinal disease were used in the series. All these animals were housed in individual cages in an air-conditioned room for thirty days prior to the experiment.

Using seven-day intervals, various determinations were made which included: alkaline phosphatase, nonprotein nitrogen, total protein with albumin:globulin ratio, bilirubin (direct and total), prothrombin times, hemoglobin, hematocrit, clotting times (six-tube method), serum protein electrophoresis (Spinco paper electrophoretic technic), and periodic microscopic urinalysis. All urine specimens were carefully collected and saved for determining the urinary excretion of nitrogen (semi-micro Kjeldahl technic), and in conjunction with nitrogen excretion data, careful weight recordings were made daily.

As in all animal experimentation, close observation and physical inspection are extremely important and in no way secondary to the other procedures carried out. Each animal was carefully observed for gross physical changes and variations from normal activity, both in and out of their cages.

RESULTS

The observations and results are presented in the following order: gross physical changes and activity variations; histopathologic changes; blood chemistry, nitrogen balance data, weight change and electrophoretic serum protein determination. Data are presented in

From the William H. Danforth Laboratory for Research in Surgery, Department of Surgery, University of Chicago, Chicago, Illinois.

* Department of Surgery; † Chairman, Department of Surgery, Stanford Medical Center, Palo Alto, California.

This work was aided by a grant from the Surgeon General's Office of the U.S. Army, Contract No. DA 49-007-MD-93.

TABLE I
Results of Eight Weeks of Protein-Free Diet
(Data are Averages of Twenty Days)

Period	Bilirubin		Pro-thrombin Times	Alkaline Phosphatase	Non-protein Nitrogen	Proteins		Hemoglobin	Clotting Time	Weekly Urinary Excretion of Nitrogen (gm.)	Weight (kg.)
	Direct	Total				Total	Albumin				
Control	0.25	0.075	7.1	6.8	20	6.35	3.65	15.4	4'8"	14.4	16.4
1st test week	0.3	0.9	7.2	7.6	23	5.7	3.20	15.5	5'10"	12.7	16.5
2nd test week	0.5	1.1	7.6	10.0	23	5.2	2.70	14.8	6'5"	7.3	17.0
3rd test week	8.5	10.4	24	5.3	2.5	14.6	6'5"	6.0	17.0
5th test week	8.5	11.5	21	4.9	1.8	14.5	7'0"	3.8	17.5
8th test week	0.7	1.5	9.0	13.6	22	4.7	0.7	14.4	9'0"	2.9	18.0

table form as an average of the results obtained from the dogs in the series (Table 1).

Gross Physical Changes and Activity Variations

In general, there was no apparent change in the physical appearance or activity in any of the animals for approximately the first four weeks, but beyond this period definite changes were observed. A progressive decrease in over-all activity was manifested in all the animals and signs of general lethargy became pronounced as the test period neared its conclusion. No attempt was made to get up or bark, which is a normal reaction at feeding time; this became a consistent observation. None of the dogs became vicious, although there was less response to petting and individual

attention. The loss of the richness in the texture of the hair, with subsequent shedding, and marked erosions at the dentogingival margins were the most marked physical changes occurring in all the dogs.

Histopathologic Findings

Most distinctive were the changes in the adrenal glands (Fig. 1). The amount of capsular and subcapsular fibroplasia was surprising. At many areas along the periphery of the cortex, projections of fibrous tissue could be seen extending through the entire cortical area to the medulla along the route of small blood vessels. Lateral extensions of this fibroplasia were seen in all three cortical zones and appeared irregularly and loosely



FIG. 1. Representative photomicrograph of the adrenal demonstrating excessive fibroplasia. Original magnification $\times 65$.

arranged. The architecture of the three zones was poorly differentiated. Along the corticomedullary zone were many cells containing large clear vacuoles and scattered diffusely throughout the adrenal cortex were cells containing lipid material taking a positive stain with Sudan III and IV.

The kidney, liver and pancreas did not demonstrate architectural distortion by fibroplasia which was as pronounced in the adrenal gland. Perhaps the most singular feature in the liver was the excessive deposition of glycogen. This was more particularly seen in the cells lining the canalicula and not quite as prominent in the more central hepatic parenchyma.

The kidneys appeared to have a minimal accumulation of fat. The glomeruli within the cortex showed no apparent fat, and the proximal convoluted tubules, although dilated, also gave no evidence of fatty change. A few of the cells had a fine pink cast in the epithelium with the Sudan stain but it was not in the droplet form that is usually seen in fat deposition. In the medulla there was also considerable dilatation of the tubules but here again no apparent change took place within the epithelium itself. A few of the tubules did show occasional fine fat droplets within the lumen.

Fat deposition was also absent from the liver; however, the excessive deposition of glycogen was very notable. The hepatic cells in general showed marked vacuolization and none of these vacuoles stained positively with Sudan III or IV.

As with the liver and kidney, the pancreas had no observable fat deposition within the pancreas proper, although there were a few large fat droplets taking the Sudan stain around some of the larger vessels. The acini and islet tissue did not appear abnormal.

Hematologic Nitrogen Balance Determinations and Serum Protein Electrophoretic Results

Changes in plasma proteins became apparent by the third and fourth weeks of the experiment. There was a marked hypoproteinemia, more particularly in the albumin fraction, and a trend toward albumin:globulin ratio reversal as early as the third week.

The clotting time (six-tube technic) was

progressively increased and an abnormal prothrombin time likewise became apparent. Hemoglobin concentration showed a consistent drop in all animals at the conclusion of the experiment. Red blood cell counts were also decreased by the seventh week, but peripheral smears showed no abnormal morphologic changes.

Alkaline phosphatase (expressed in Bodansky units) showed varying degrees of elevation, but its accuracy to reflect the true extent of hepatic damage or involvement has never been too readily accepted in past biological experimentation in this laboratory. Non-protein nitrogen levels remained within normal limits and the values obtained did not reflect renal disease. There was a small increase in both the direct and total bilirubin measurements in all animals by the end of the test period.

The weights before the institution of the test diet were maintained and this can be accounted for in part by the adequate caloric intake and the progressive decrease in physical activity.

As was to be expected, urinary excretion of nitrogen became greatly reduced, and at the terminal part of the experiment, the small amount of nitrogen excreted undoubtedly represented protein breakdown from the animal's own tissues.

Electrophoretic patterns of serum proteins were determined on the tenth, twentieth, thirtieth, fortieth and fiftieth test days. There was a rapid decrease in the albumin peak. This was seen electrophoretically by the tenth test day. The *alpha*-globulins showed a decrease by the twentieth day, while the *beta*- and *gamma*-globulin fractions did show a reduction in total components by the fortieth test day.

The electrophoretic findings corresponded quite well with the blood and plasma values which were drawn at approximately corresponding times. Total proteins showed a progressive fall, initially due to a reduction in the albumins, but at the end of the fifty-six-day test period there was also a decrease in the globulin fraction.

COMMENTS

The histologic findings of minimal or no fat in the tissues and organs reviewed was a con-

sistent observation with the exception of the adrenal gland.

In contrast, an excessive glycogen deposition was seen in all of the specimens, especially in the liver. The hepatic cells which showed extreme vacuolization did not stain with Sudan III or IV.

The adrenals, histologically, gave the most marked histologic change (Fig. 1). Fibroplasia plus fat and glycogen deposition were present. Cortical cellular patterns in the three zones were so disrupted that differentiation in most instances was very difficult and hydropic degeneration with large vacuolated cells (not staining with Sudan III or IV) were present along the entire corticomedullary junctions.

CONCLUSION

The histopathologic findings would in our estimation point to a hypoadrenal state with irreversible damage to the adrenals. The animals' ability to handle general stress conditions such as disease or rapid environmental changes would seem, in view of these facts, to be greatly impaired; the diet as given causes an excessive deposition of glycogen in the tissues but little fat; the significance of the appearance of diffuse hepatocellular vacuo-

lization aside from vacuolization caused by glycogen deposition remains to be determined; although there was not a marked increase in prothrombin times, a small but definite elevation in all determinations was observed at the conclusion of the experiment. What abnormality, if any, in liver function is reflected in this finding was unfortunately not pursued in this particular project.

ACKNOWLEDGMENT

We are deeply indebted to Dr. Eleanor M. Humphreys, Professor Emeritus of Surgical Pathology, University of Chicago, for her interpretation and review of all the histopathologic sections.

REFERENCES

1. PFAFF, W. W., STEMMER, E. A., AL-SHAMMA, A., DAWSON, D. and ALLEN, J. G. The treatment of pooled human plasma with formaldehyde at 60°C. for ten hours. *S. Forum*, 9: 1959.
2. DAWSON, C., AL-SHAMMA, A., PFAFF, W. W. and ALLEN, J. G. Heat tolerance and biologic usefulness of plasma treated with chloroacetate and formaldehyde. *Fed. Proc.*, 18: 131, 1959.
3. ALLEN, J. G., STEMMER, E. A. and HEAD, L. R. Similar growth rates of litter mate puppies maintained on oral protein with those on same quantity of protein as daily intravenous plasma for 99 days as the only protein source. *Ann. Surg.*, 144: 349, 1956.

