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Influence of a Pancreas Extract and Other Proteins on Liver Fat and Ketosis.

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Some years ago while carrying out experiments on the relation of the preceding diet to the extent of the fasting ketosis in albino rats we were surprised when a much greater ketonuria was observed with animals which had been for a few days on a diet composed of butter 65 and glucose 35, than one in which the glucose was replaced by casein. We now know that this was associated with the fatty livers which are produced by low protein diets.[1, 2, 3] It has been found[4] that the fat leaves these livers more quickly when the rats are fasted if they are given a pancreas extract which Dragstedt, *et al.*, have shown[5] prevents the deposition of fat in the livers of depancreatized dogs maintained on insulin and which, believing it to be a new fat metabolizing hormone, they have named *lipocaic.* We were primarily interested in the influence on ketosis of the more rapid loss of fat from the fatty livers on fasting.

In Experiment 1 the rats had been on the low protein diet[1] for 10 days before being fasted. Urine collections were made daily, nitrogen determinations carried out by the macro Kjeldahl method and total ketones determined by Van Slyke's well known method. The pancreas extract was prepared according to Dragstedt's directions.[5] The solution administered in Experiment 1 contained 48.2% total solids and 5.5% nitrogen. There was a marked diminution in the degree of ketonuria as the dose of pancreas extract was increased. A ketonuria was produced in Experiment 2 by feeding each rat 3 cc. per day of a mixture half butter fat and half cotton seed oil and in Experiment 3 by the method of Butts and Deuel[6] using sodium caprylate.[7] One and a half cc. of 5% solution was adminis-

1 Channon, H. J., and Wilkinson, H., *Biochem. J.*, 1935, **29**, 350.

2 Best, C. H., and Channon, H. J., *Biochem. J.*, 1935, **29**, 2651.

3 Beeston, A. W., Channon, H. J., and Wilkinson, H., *Biochem. J.*, 1935, **29**, 2659.

4 MacKay, E. M., *Am. J. Physiol.*, 1937, **119**, 783.

5 Dragstedt, L. R., Van Prohaska, J., and Harms, H. P., *Am. J. Physiol.*, 1936, **117**, 175.

6 Butts, J. S., and Deuel, H. J., Jr., *J. Biol. Chem.*, 1933, **100**, 415.

7 Butts, J. S., Cutler, C. H., Hallman, L., and Deuel, H. J., Jr., *J. Biol. Chem.*, 1935, **109**, 597.

tered to each rat by stomach tube twice daily. In both experiments the ketosis was reduced by the pancreas extract containing in this case 31.5% solids and 3.8% nitrogen. We have found that other factors which rapidly reduce a high liver fat during fasting[8] also reduce the degree of ketosis as measured by the ketonuria.[9] Deuel, *et al.*,[10] have found that although choline causes the fat to leave the liver rapidly it does not reduce the ketosis very much.

There is some question as to whether or not Dragstedt's preparation[5] contains a new lipotropic substance. Presumably he ruled out the lecithin or choline content as being responsible.[11] Unfortunately histological instead of chemical methods were used for estimating liver fat. In comparing raw or autoclaved pancreas with choline equivalent to that which it contained in its influence upon the liver fat when raised by a low protein diet Aylward and Holt[12] came to the conclusion that the effect was similar. An examination of their data shows that the amount of liver fat in relation to body size is usually considerably greater when choline was given than after an equivalent amount of pancreas. However, Best[13] has found that the lipotropic effect of Dragstedt's *lipocaic*[5] in rats with fatty livers can be accounted for by the choline plus the protein content. The experiments in Table II have a bearing upon this point. Liver "fat" was determined by saponification, acidification and extraction with petroleum ether. It therefore represents the total fatty acids plus nonsaponifiable lipids. The pancreas extract (Exp. 6) was much more potent than either casein or the crude proteins of skeletal muscle (beef) in preventing the deposition of fat in the liver. When included in the diet in sufficient quantity the pancreas extract caused the liver fat to remain approximately normal in amount (Exp. 7).

In Experiment 8 a rough separation of the protein and possible choline content of the pancreas extract was made. Five hundred and fifty grams of the pancreas extract used in Experiment 1 was added to water with a final volume of 1100 cc. This is noted on the last column in Table II as pancreas extract A and the solution contained 24.1% total solids and 2.75% nitrogen. Eight hundred cc.

8 MacKay, E. M., *Am. J. Physiol.*, 1937, **120**, 361.

9 MacKay, E. M., and Barnes, R. H., *Am. J. Physiol.*, 1937, **118**, 184.

10 Deuel, H. J., Jr., Murray, S., Hallman, L. F., and Tyler, D. B., *J. Biol. Chem.*, 1937, **120**, 277.

11 Van Prohaska, J., Dragstedt, L. R., and Harms, H. P., *Am. J. Physiol.*, 1936, **117**, 166.

12 Aylward, F. X., and Holt, L. E., Jr., *J. Biol. Chem.*, 1937, **121**, 61.

13 Best, C. H., personal communication.

TABLE I.

Experiments 1, 2 and 4—averages of groups of 4 females each.
Experiments 3 and 5—averages of groups of 4 males each.

Exp.	Group	Body Wt., gm.	Body Surface, sq.cm.	Urine ketones in mg. per sq. dcm. B.S. per day					Urine nitrogen in mg. per sq. dcm. B.S. per day					Dose per 100 sq. dcm. Body Surface per day		
				1	2	3	4	5	1	2	3	4	5	*Pancreas Extract mg.	Nitrogen mg.	Casein mg.
1	1	144	311	2	26	15			18	21	14			0	0	
	2	141	306	2	15	19			21	17	16			32	4	
	3	139	304	2	14	16			14	17	14			46	5	
	4	139	304	2	15	11			23	19	20			80	9	
	5	144	311	1	0	3			26	29	27			158	18	
	6	139	304	0	0	1			30	32	35			316	36	
2	1	186	370	2	5	5	2	3	23	19	21	14	14	0	0	
	2	191	377	1	1	0	0	1	20	35	27	23	25	195	24	
3	1	262	464	2	8	11	27	10	39	25	25	21	23	0	0	
	2	261	463	3	3	1	4	1	37	35	28	27	30	159	19	
4	1	181	363	1	8	15	14	12								0
	2	172	351	2	10	16	12	9								25
	3	171	349	1	15	10	4	8								50
	4	171	349	0	1	0	0	0								100
5	1	250	450	5	16	10	8	12								0
	2	249	449	1	12	8	6	4								25
	3	251	451	2	10	2	4	6								50
	4	252	452	0	1	0	1	0								100

*Calculated here on a dry basis but actually administered in solution.

TABLE II.

Averages for 6 female rats in each group. Initial weight when removed from stock diet. Final weight after 12 days on the low protein, high fat diet. Except Exp. 6 which had 4 male rats in each group and were on the diet 7 days.

Exp.	Group	Body Wt. Initial gm.	Body Wt. Final gm.	‡Body Surface sq.cm.	Liver Wt. Actual gm.	Liver Wt. Mg. per sq. dcm. Body Surf.	Liver Fat %	Liver Fat mg. per sq. dcm. Body Surf.	†Food Intake in grams per sq. dcm. B.S. per day *Special diet	Pancreas Extract	Casein	Skeletal Muscle	Pancreas Extract cc. per sq. dcm. B.S. per day
6	1	177	187	371	7.42	2000	8.2	164	2.29				
	2	167	180	362	6.49	1795	4.1	74	2.05	.18			
	3	173	173	352	6.42	1825	5.6	102	1.80	.08			
	4	173	173	352	6.79	1935	5.9	114	2.15	.04			
	5	175	181	363	6.55	1805	6.9	125	2.37	.02			
	6	167	175	355	6.80	1920	6.6	127	1.97		.17		
	7	184	191	377	7.33	1945	7.3	142	1.78		.07		
	8	171	180	362	7.83	2165	8.2	178	2.22		.04		
	9	180	180	362	6.86	1900	8.9	169	2.18		.02		
	10	180	175	355	6.74	1900	7.8	148	2.15			.19	
	11	180	185	369	6.93	1880	8.4	158	2.32			.10	
	12	180	185	369	7.01	1900	8.1	154	2.30			.05	
	13	177	173	352	6.75	1930	8.8	170	2.26			.02	
7	1	157	138	304	5.84	1920	14.7	282	1.97				
	2	144	143	309	5.86	1865	4.1	76	2.00	.22			
	3	152	144	311	5.95	1915	3.9	75	1.56	.34			
	4	144	150	321	6.86	2135	7.6	162	1.80	.16			
	5	142	151	322	6.27	1945	6.3	123	1.51	.30			
8	1	139	135	299	5.15	1720	6.7	115	2.66				0
	2	138	136	301	4.92	1635	7.4	121					0
	3	137	142	308	5.52	1785	3.1	55	2.23				1A
	4	139	142	308	6.07	1970	3.5	69					2A
	5	148	157	330	5.80	1755	3.3	58	2.29				1B
	6	139	150	321	6.15	1920	3.2	61					2B
	7	139	142	308	5.50	1780	3.5	62	2.05				1C
	8	141	146	314	5.38	1710	3.7	63					2C

*The same low protein, high fat diet as used in other experiments, butter fat 40, glucose 45, brewers yeast 5, vitamin free casein 5, and Osborne and Mendel's salt mixture 5.

†For 12-day period on the special diet, with and without additions. The body surface used was calculated from the average body weight (Carman, G. G., and Mitchell, H. H., *Am. J. Physiol.*, 1928, **76**, 381) of the 12-day period. The pancreas extract is calculated on a water-free basis, the casein was vitamin free and the skeletal muscle was dried powdered lean beef.

‡Body surface calculated on final weight for liver figures.

of this were poured into 16 liters of 95% ethyl alcohol with vigorous stirring and a white colloidal precipitate was formed. This settled out over night forming a brown paste with a deep yellow supernatant liquid above which was filtered off. The paste, free of practically all the choline it might contain but which represents the major portion of the protein in the pancreas extract, was dried so that it was free of alcohol and water added to a final volume of 400 cc. which was pancreas extract B. It contained 40.2% solids and 4.01% nitrogen. The supernatant liquid was evaporated to dryness and the residue taken up in water so that the final volume was 400 cc. making extract C. It would contain the bulk of any choline in the extract. The solids and nitrogen content were 7.0% and 1.51% respectively. The two fractions (extracts B and C) were about equally effective in preventing the accumulation of fat in the liver. This result supports Best's contention[18] that both the protein and choline contained in the pancreas extract act to prevent the deposition of fat in the liver of the rat when on a diet low in protein and choline but high in fat.

Since choline does not appreciably affect the ketosis of fasting fatty liver rats[10] it was desirable to compare an equivalent amount of protein with the pancreas extract in its antiketogenic effect. Casein was selected for this purpose and in Experiment 4 can be seen to be equally as effective as the same amount of pancreas extract (both on a dry weight basis) in antiketogenic activity. In this experiment the ketosis was produced in the same manner as in Experiment 1, the rats having been on the fatty liver producing diet for 10 days before fasting was commenced.

Summary. Although they do not offer direct proof our experiments support the view that the reduction in liver fat caused by the pancreas extract which Dragstedt has described as *lipocaic* is due to the sum of the lipotropic effects of the choline and protein which it contains. The influence of the pancreas extract upon the fat content of the livers of rats on a low protein and low choline but high fat diet is greater than that of a similar amount of protein in other forms. The ketosis which results when rats with such fatty livers are fasting is reduced about equally by the pancreas extract and an equivalent amount of casein. Althought both the choline and the protein contained in the pancreas extract affect the fat content of the liver, choline is without antiketogenic activity under the conditions studied here.